

Ion Effects on Metabolism in the Adult Mammalian Brain *in vitro*

Evidence of a Potassium-Induced
Stimulation of Active Uptake of
KCl into Neuroglial Cells

by

LEIF HERTZ



FADLs FORLAG
KØBENHAVN · ÅRHUS · ODENSE
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Denne afhandling er af det natur- og lægevidenskabelige
fakultetsråd ved Odense Universitet antaget til offentligt
at forsvares for den medicinske doktorgrad.

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Preface

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- A. Hertz, L. & M. Schou: Univalent cations and the respiration of brain-cortex slices, Biochem. J. 1962, 85: 93-104.
- B. Hertz, L. & T. Clausen: Effects of potassium and sodium on respiration: their specificity to slices from certain brain regions, Biochem. J. 1963, 89: 526-533.
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- H. Arnfred, T., L. Hertz, L. Lolle & H. Lund-Andersen: An improved holder for transfer of brain slices during in vitro incubation, Exp. Brain Res. 1970, 11: 373-375.
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- K. Arnfred, T. & L. Hertz: Effects of potassium and glutamate on brain cortex slices: Uptake and release of glutamic and other amino acids, J. Neurochem. 1971, 18: 259-265.
- L. Booher, J., L. Hertz & Z. Lodin: A simplified technique for cultivation of dissociated neurons and glial cells in the Rose chamber, Neurobiology 1971, 1: 27-31.
- M. Booher, J., H. Fosmark & L. Hertz: Rates of oxygen uptake by dorsal root neurons cultivated in the Rose chamber in well-oxygenated medium or in relative hypoxia, Neurobiology 1971, 1: 32-36.
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Contents

I.	INTRODUCTION	11
A.	AIM OF PAPER	11
B.	COMPLEXITY OF BRAIN	12
1.	Cell types	12
2.	Extracellular space	14
3.	Techniques used for study of metabolism in individual cell types	15
4.	Ontogenetic and phylogenetic development	18
5.	Compartmentation	20
6.	Subcellular fractionation	21
II.	OXYGEN CONSUMPTION	22
A.	RATE OF OXYGEN UPTAKE BY BRAIN	22
1.	Interspecies variation	22
2.	Topographical variation	22
3.	Variation between different modes of preparation	25
a.	Intact brain	25
b.	Slices	25
c.	Isolated cells	25
d.	Homogenates and cell suspensions	29
B.	ION EFFECTS ON OXYGEN CONSUMPTION	29
1.	Maintenance of respiration	29
a.	Slices	29
	Sodium	30
	Potassium	31
	Electrical stimulation	31
	Calcium and magnesium	32
b.	Isolated cells	32
c.	Homogenates	34
2.	Stimulation of respiration	34
a.	Slices	34
	Potassium and related monovalent cations	34
	Other stimulating procedures	36
	Calcium and magnesium	36
	Sodium	37
	Drug inhibition	37
	Tissue specificity	38
	Topographical specificity within the nervous system	39
b.	Homogenates and sieved cells	39
c.	Isolated cells	40
d.	Subcellular particles	40
3.	pH effects on stimulated and unstimulated respiration ..	42
4.	Anion effects	42
C.	DISCUSSION	43

III. PHOSPHATE METABOLISM	45
A. PHOSPHATE METABOLISM IN BRAIN	45
1. Phosphorus fractions	45
a. Acid-soluble phosphorus	45
b. Acid-insoluble phosphorus	45
2. Phosphate metabolism in vivo	46
3. Phosphate metabolism in vitro	46
B. ION EFFECTS ON PHOSPHATE METABOLISM	47
1. Sodium	47
a. Acid-soluble phosphorus	47
b. Acid-insoluble phosphorus	47
2. Potassium	47
a. Acid-soluble phosphorus	47
b. Acid-insoluble phosphorus	48
3. Other cations (ammonium and calcium)	50
4. Electrical stimulation	51
a. Acid-soluble phosphorus	51
b. Acid-insoluble phosphorus	51
5. Drugs	52
6. Amino acids and transmitters	52
C. DISCUSSION	53
IV. ION DISTRIBUTION AND TRANSPORT	55
A. ION TRANSPORT AND CONTENT	55
1. Correlation between ion contents and membrane polarization	55
2. In vivo	56
a. Ion content	56
b. Ion transport	57
c. Changes in ion content and transport	57
d. Extracellular and intracellular spaces	57
e. Membrane potentials	58
f. Spreading depression	59
3. In vitro	60
a. Spaces	60
b. Swelling	62
c. Ion concentrations	64
Potassium	64
Sodium	67
Chloride	67
d. Membrane potentials	68
e. Ion transport	69
Potassium	70
Sodium	72
Chloride	72
B. ION EFFECTS	73
1. Spaces	73
2. Swelling	74
a. Sodium	74
b. Potassium	75
c. Glutamate and related compounds	76
d. Other anions	76

e.	Electrical stimulation	77
3.	Ion concentrations	77
a.	Sodium concentration in the tissue	77
	Sodium concentration in the medium	77
	Potassium concentration in the medium	77
	Electrical stimulation	78
	Glutamate	79
	Calcium and magnesium in the medium	79
b.	Potassium concentration in the tissue	79
	Sodium concentration in the medium	79
	Potassium concentration in the medium	80
	Calcium and magnesium in the medium	82
	Glutamate	83
	Electrical stimulation	84
	Drugs	84
c.	Chloride concentration in the tissue	84
d.	Calcium concentration in the tissue	85
4.	Membrane potential	85
a.	Potassium	85
b.	Electrical stimulation	88
c.	Glutamate and related compounds	88
d.	Sodium	89
e.	Calcium and magnesium	89
5.	Ion transport	89
a.	Potassium effects	89
	Potassium fluxes	90
	Sodium fluxes	90
	Calcium fluxes	91
	Chloride fluxes	92
b.	Sodium effects	92
c.	Electrical stimulation	92
	Potassium fluxes	92
	Sodium fluxes	93
d.	Glutamate effects	94
C.	DISCUSSION	94
V.	NITROGEN METABOLISM	99
A.	AMINO ACIDS, TRANSMITTERS, PROTEIN AND NUCLEIC ACIDS IN BRAIN	99
1.	Interrelations between ions and nitrogenous compounds	99
2.	Amino acids and their derivatives in brain	99
a.	In vivo	99
b.	In vitro	102
B.	ION EFFECTS ON NITROGENEOUS COMPOUNDS	106
1.	Exchange between tissue and medium	106
a.	Measurement of ion effects on amino acid fluxes and metabolism	106
b.	Sodium effects	107
c.	Potassium effects	107
d.	Electrical stimulation	111
e.	Glutamate effects	112
f.	Acetylcholine effects	112

g. Calcium and magnesium effects	112
2. Metabolism	113
a. Potassium effects	113
b. Electrical stimulation	115
c. Calcium	115
d. Sodium	115
C. DISCUSSION	115
VI. INTERMEDIARY METABOLISM	118
A. BRAIN METABOLISM	118
1. Evaluation of intermediary metabolism	118
2. In vivo	118
3. In vitro	119
a. Pathways	119
b. Substrates	119
Absence of a substrate	123
Glucose, fructose, pyruvate and lactate	123
Citrate, α -ketoglutarate, succinate, fumarate and malate.....	123
Oxaloacetate	124
Glutamate, glutamine and GABA	125
Acetate	126
c. Intermediates	126
B. ION EFFECTS ON BRAIN METABOLISM.....	127
1. Pathways	127
2. Substrates	127
a. Absence of substrate	127
b. Glucose, fructose, pyruvate and lactate	128
c. Citrate, α -ketoglutarate, succinate and fumarate...	128
d. Oxaloacetate	129
e. Glutamate and GABA	129
f. Acetate	129
3. Intermediates	129
a. Sodium effects	129
b. Potassium, glutamate and electrical stimulation ...	130
c. Calcium and magnesium	132
4. Potassium-activated enzyme reactions	132
a. Pyruvate kinase	132
b. Acetylcoenzyme A synthetase	133
c. $\text{Na}^+ - \text{K}^+ - \text{ATPase}$	133
C. DISCUSSION	137
VII. CONCLUSION	140
SUMMARY	142
DANSK RESUMÉ (SUMMARY IN DANISH)	144
REFERENCES	146
INDEX	207

Introduction

A. AIM OF PAPER

Deviations in the concentrations of monovalent and divalent cations (e.g. H^+ , Na^+ , K^+ , Li^+ , Cs^+ , Rb^+ , NH_4^+ , choline $^+$, Ca^{++} and Mg^{++}) from those occurring in plasma (i. e. around 120 mM Na^+ , 5 mM K^+ , 1 mM Ca^{++} , 1 mM Mg^{++} and $10^{-7.3}M H^+$) are known to affect the metabolism of brain tissue. The metabolic actions evoked by such ionic deviations will be dealt with in the following review, in which the term metabolism is to be regarded in its broadest sense. The influence exerted by the ions on the rate of energy-yielding processes (II, III and VI) will accordingly be discussed together with that on energy-requiring processes such as the transport of monovalent cations (IV) and the turnover of certain amino acids (V). Correlations will be sought between ion effects on the various aspects of metabolism, and evidence will be presented supporting the concept that the effects exerted on energy metabolism are directly evoked by those on some of the energy-requiring processes. To this end the substrate requirements (VI) of the different phenomena will be compared together with their degree of specificity to certain anatomical and histological structures. The study of the latter is complicated by the complexity of brain tissue, which is discussed in I B.

Main emphasis will be laid on in vitro investigations, and unless otherwise stated the observations quoted refer to the metabolism of preparations from the adult, mammalian brain during incubation in oxygenated media containing glucose as the substrate. The reason to focus minor attention on phylogenetically or ontogenetically less developed preparations is the absence of such characteristic phenomena as the potassium-induced augmentation of oxygen consumption (II B, 2a) and swelling (IV B, 2b) in brain cortex slices from, e.g. new born rats as well as the great alterations in brain histology during both ontogenetic and phylogenetic development (I B, 4).

B. COMPLEXITY OF BRAIN

1. Cell types

The adult, mammalian brain is both anatomically and histologically an extremely heterogeneous tissue. The gross anatomical complexity impedes experimental investigations to only a minor degree since e. g. slices may be obtained from several structures in suitable species (cf. II A, 2). The histological heterogeneity, on the other hand, probably at present represents the major obstacle for experimental work on brain biochemistry as illustrated by the fact that certain procedures may lead to oppositely directed changes in the different cell types (see, e. g. Hydén, 1967a). Two main elements, i. e. nerve cells and neuroglial cells, constitute the bulk of the tissue, but each of these may on morphological grounds be divided into several subgroups.

The nerve cells may be classified according to the size and shape of their cell bodies (perikarya) or according to the number, length and mode of branching of their processes. Virtually nothing is known about possible biochemical differences between different types of neurons. The nerve cell perikarya account for only about 5-10 % of the volume in mammalian brain cortex (Economo, 1926; Peters & Flexner, 1950; Rebhan, 1956; Haug, 1956; Haug, 1960; Schädé & Baxter, 1960). The volume occupied by their processes is, however, probably several times greater, and it has been estimated that the proximal and distal dendrites account for about one-fourth of the total volume of the grey matter (Schädé & Baxter, 1960; Schädé, Backer & Colon, 1964).

The glia cells are traditionally divided into fibrous and proto-plasmatic astrocytes, oligodendrocytes and microglia. In grey matter, the characteristic neuroglial cells are the protoplasmatic astrocyte (Cajal, 1909; Wolff, 1970) and the oligodendrocyte, whereas the fibrous astrocyte is found mainly in white matter. The volume occupied by easily recognizable glia cell bodies is relatively small, though the glia cells may outnumber the nerve cells by a factor of about five to ten (Nurnberger & Gordon, 1957; Pope, 1958; Hild, 1964; Johnston & Roots, 1972).

Thus a considerable fraction of the tissue volume remains to be accounted for, and in grey matter the main part of this fraction is made up by the so-called "neuropil". This term refers to a rather badly defined, intricately interwoven network of minute neuronal and neuroglial processes, which (Fig. I, 1) taper into lamellar sheets of 200-1000 Å thickness (Pappas & Purpura, 1961; Wolff, 1965). The ratio between glia cells and nerve cells in the "neuropil" is uncertain and probably varies in the different parts of the nervous system. In the hippocampal cortex - from which Fig. I, 1 was drawn - astroglia cells thus seem to constitute only 5-6 % (Blackstad, 1967), whereas Wolff (1970) found 25-30 % astrocytes in the occipital cortex of the

rat, and Hydén (1967b) estimated that about nine-tenths of the Deiters' cell surroundings are made up by glia cells. The previously mentioned observation by Schädé and coworkers (1960, 1964) that the dendrites altogether occupy about one-fourth of the brain cortex, supports the concept that above half of its volume may be occupied by glia cells (cf. also Schultz, Maynard & Pease, 1957). The volume occupied by the nerve endings has been estimated to at most 15 % of the brain (Salganicoff & Koeppe, 1968).



Fig. I, 1. Line drawing of an electron micrograph of stratum radiatum of regio superior of the rat hippocampal cortex. The micrograph was one of a series facilitating identification of the structures. Scale is 1μ .

D, Dendritic shaft or larger branch; d, finer dendritic branch; s, spine; t, axon swelling with synaptic vesicles; g, astroglial process; ▲, axon.

From Westrum & Blackstad, 1962.

It is a common impression that the glia cells contain fewer mitochondria than the nerve cells (e.g. Windle, 1958, p. 64; Abood, 1969), but other authors have described a relatively abundant presence of mitochondria in at least some types of glial cells (see, e.g. Wyckoff & Young, 1956; Farquhar & Hartmann, 1957; De Robertis & Gerschenfeld, 1961; Mugnaini & Walberg, 1964). The most reliable quantitative estimate is probably that obtained by Hamberger, Blomstrand & Yanagihara (1971) who reported a mitochondrion content of 14 % in their neuron-enriched fraction versus 7 % in the glial-enriched fraction (cf. 1 B, 3).

2. Extracellular space

Besides the two main cellular elements the brain contains an extracellular compartment, and for years a controversial question has been the magnitude of the extracellular space (cf. IV A, 2d). Basing their conclusions on electron micrographs, many investigators (e.g. Horstmann & Meves, 1959; Kuffler & Potter, 1964) have arrived at an estimate that only about 5 % of the total volume in the brain cortex is occupied by extracellular fluid. This fluid is mainly localized in intercellular clefts of about 150 Å in width. On the other hand, Van Harreveld and coworkers have reported that the extracellular space is considerably larger in vivo, but shrinks during the period between the arrest of the circulation and conventional fixation (Van Harreveld, 1962). This concept is supported by their finding that a special fixation technique yields an extracellular space of about 20 % (Van Harreveld, Crowell & Malhotra, 1965; cf. however also Kuffler & Nicholls, 1966; Ibatá, Piccoli, Pappas & Lajtha, 1971). This space is mainly found between non-myelinated axons, whereas other cellular elements (and thus also neurons and glial cells) are separated by the narrow slits described above (Van Harreveld & Malhotra, 1967). Accordingly, compounds (e.g. ions) which are released from cells may in any case become confined to such a small volume that their concentrations become high enough to exert biochemical effects on adjacent cells (cf. Hertz, 1973). As a further complication for evaluation of ion content and transport in the extracellular space the presence of an intercellular anionic substance has been reported (Pease, 1966; Bondareff, 1966, 1967). Conceivably some binding of cations may occur to this substance (cf. Adey, 1967), or the structuration of the space may cause a reduction or an increase (cf. Metzner, 1965) of diffusion rates. The demonstration of ATP-ase activity in the extracellular space of brain and retina (Torack & Barnett, 1963; Marchesi, Sears & Barrnett, 1964; Torack, 1965; O'Daly, 1967) may even indicate capacity for active transport (cf. VI B, 4c).

3. Techniques used for study of metabolism in individual cell types

The histological heterogeneity of brain tissue has necessitated the development of methods to separate its constituent cells from each other. By aid of microdissection (Lowry, 1953, 1957; Hydén, 1959; 1967a) it is possible to obtain single nerve cells or clumps of glia cells, which are exceedingly well separated from each other as can be seen in Fig. I, 2. A drawback of the method is that the nerve cells of necessity lose part of their dendrites, which is then a source of contamination in the glia cell samples (Hydén & Lange, 1961; Hamberger, 1963; Kuffler & Nicholls, 1966). It must also be emphasized that the experiments on isolated glia cell clumps do not allow any distinction between metabolic processes occurring inside the glia cells and those occurring in the intercellular space (cf. I B, 2 and Adey, Kado, Didio & Schindler, 1963; Adey, 1967). Damage of the membranes has been observed (Roots & Johnston, 1964; Johnston & Roots, 1965), but this criticism was rejected by Hydén (1967a), and metabolically the samples seem surprisingly intact (cf. II A, 3c). Cells extracted from cultures grown under suitable conditions (Fig. I, 3) may similarly show a well maintained energy metabolism (Hartman, Hertz, Fosmark & Lodin, 1970; Schousboe, Booher & Hertz, 1970; Booher, Fosmark & Hertz, 1971; Dittmann, Hertz, Fosmark, Sensenbrenner & Mandel, 1973; Dittmann, Hertz, Schousboe, Fosmark, Sensenbrenner & Mandel, 1973; Dittmann, Sensenbrenner, Hertz & Mandel, 1973), and cultivated glial cells and neurons have been useful in several biochemical and physiological studies (e.g. Hild, Chang & Tasaki, 1958; Hild, 1964; Sensenbrenner, Lodin, Treska, Jacob & Mandel, 1968 - cf. also Fig. VI, 3).

Isolated glia and nerve cell fractions (Fig. I, 4) may be obtained in larger amounts using differential centrifugation (e.g. Roots & Johnston, 1964; Rose 1965b, 1967; Bradford & Rose, 1967; Azcurra, Lodin & Sellinger, 1969; Rose & Sinha, 1969; Blomstrand & Hamberger, 1969, 1970; Norton & Poduslo, 1970; Blomstrand, 1971). Also these preparations may show considerable metabolic and physiologic activity (e.g. II A, 3c and IV A, 3c), though they in all probability remain less intact than the microdissected cells (Dittmann et al., 1973c). By use of the separation techniques, by investigations on tumor tissue (e.g. Victor & Wolf, 1937) or by aid of histological (e.g. Diamond, Law, Rhodes, Lindner, Rosenzweig, Krech & Bennett, 1966) and autoradiographic (Altman, 1963; Droz & Leblond, 1963) techniques a good deal of information has been obtained about metabolism and function of the individual brain cells. It must, however, also be kept in mind that each of these procedures has its own - often serious - limitations (see, e.g. the criticism raised against the Rose differential centrifugation technique by Cremer, Johnston, Roots & Trevor (1968) and against the use of tumor tissue by Utley (1963)). This may ex-

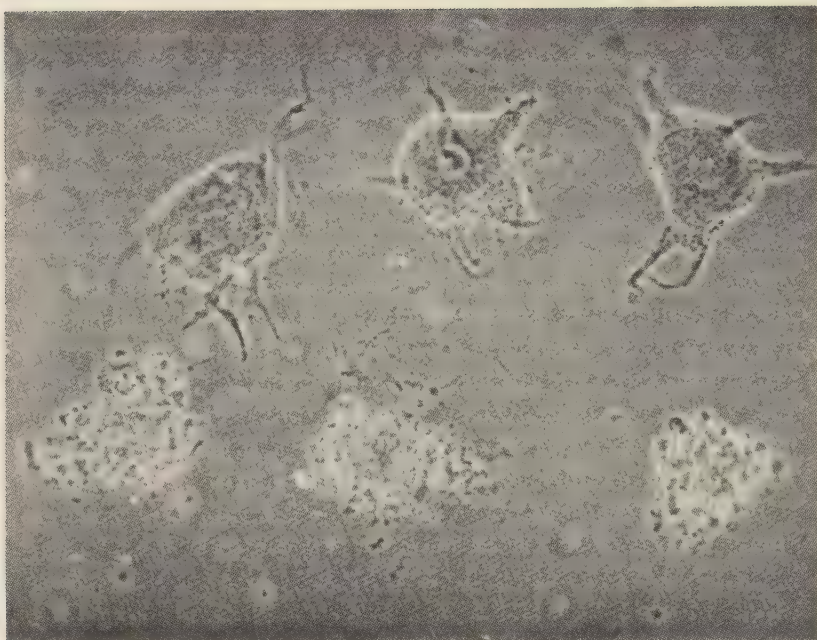
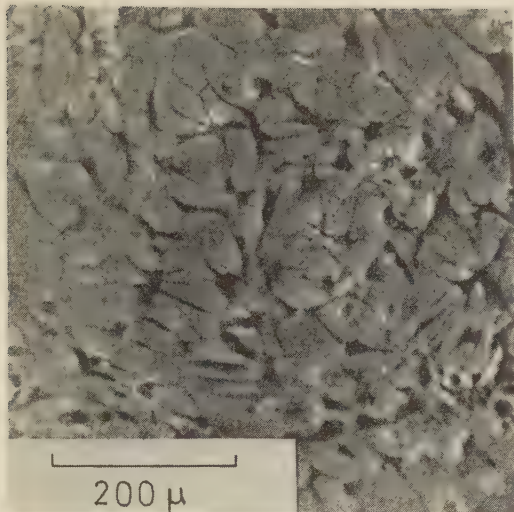


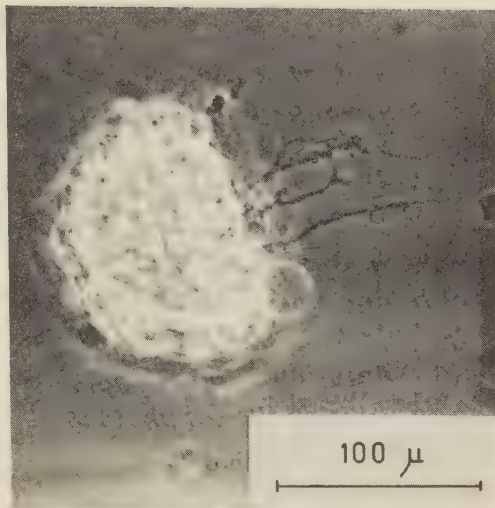
Fig. I, 2. Upper row. Three Deiters' nerve cells dissected freehand, cleaned from the surrounding glia, and photographed in the phase contrast microscope. Lower row. Three collections of glia cells, each of which originally surrounded the nerve cell situated above.

From Hydén, 1967a.

plain the often conflicting results and emphasize the need of attacking the same problem with several different techniques (cf. also Hertz, 1969).



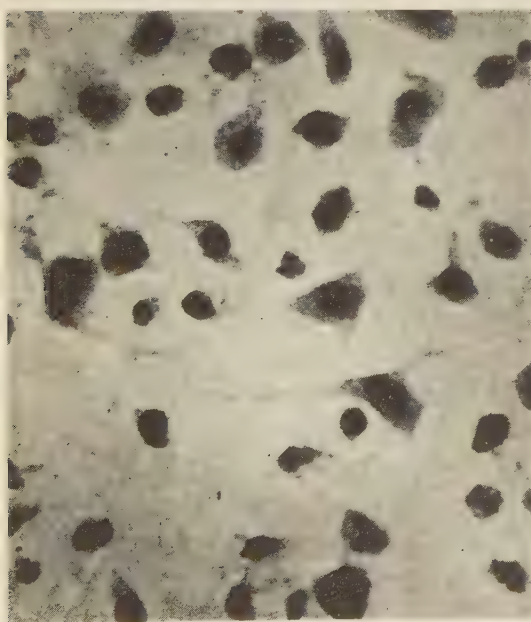
a.



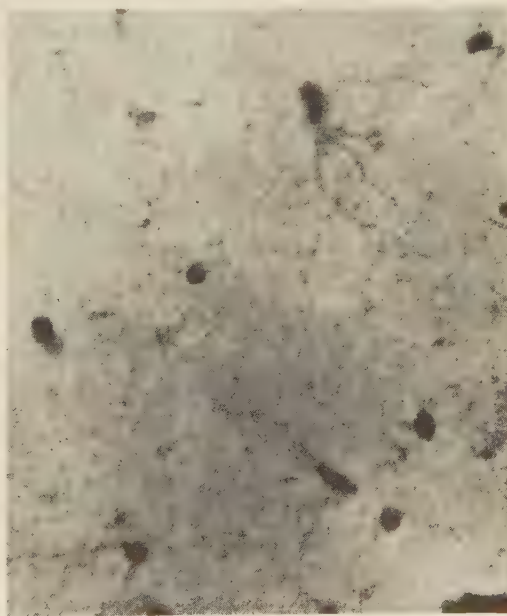
b.

Fig. I, 3. Phase contrast micrographs of (a) migrated astrocytes from the spinal cord of a 10-day-old chick embryo after 33 days of cultivation in a Rose chamber and (b) nerve cell clump from the dissociated cerebral hemispheres of the chick embryo after 7 days of cultivation in a Falcon flask. Note homogeneity of cells.

(a) from Booher, Hertz & Lodin, 1971 and (b) from Dittmann *et al.*, 1973c.



a.



b.

Fig. I, 4. Nerve cell (a) and glia cell (b) fraction obtained by gradient centrifugation of sieved rabbit cerebral cortex. Papanicolau stain.

From Blomstrand & Hamberger, 1969.

4. Ontogenetic and phylogenetic development

Another approach to the study of differences between nerve cells and glia cells is the use of immature animals or of phylogenetically less developed species. Even between relatively closely related mammals, great temporal differences occur in brain development as reflected by the different time periods for maximum DNA synthesis. In the rat brain the rate of DNA synthesis is high during the first 2-3 weeks after birth (Mandel, Rein, Harth-Edel & Mardell, 1964; Fish & Winnick, 1968; Mori, Yamagami & Kawakita, 1970; Schousboe, 1972), whereas cell proliferation ceases earlier in the guinea pig (Mandel et al., 1964). The late synthesis of DNA seems predominantly to represent a proliferation of glia cells, which in the rat brain cortex mainly or exclusively are formed postnatally (Altman, 1969). Histochemical studies have shown that the enzymatic activities of the astrocytes remain low longer than those of the oligodendrocytes (Franck, Schoffeniels & Gereb-zoff, 1970). The number of neurons per volume brain cortex decreases during post-natal ontogenesis (Brizee & Jacobs, 1959a, b; Brizee, Vogt & Kharetchko, 1964; Vernadakis & Woodbury, 1965; Tower & Bourke, 1966; Franck, 1970), but the development of the dendrites occurs at an ontogenetically relatively late stage (e.g. Schädé & Baxter, 1960 - cf. also Altman, 1967). It is thus difficult with certainty to correlate the onset of metabolic events with maturation of either dendrites or neuroglia (cf. V C) though there seems to be a rather abrupt termination of DNA synthesis, i.e. glia cell proliferation about the 15th postnatal day in the rat (Fig. I, 5; Schousboe, 1972), whereas the development of the processes may occur more gradually during the whole first month (Fig. I, 6; Eayrs & Goodhead, 1959; Aghajanian & Bloom, 1967). As a further complication, differences may, however, occur between the rate of development in the same species kept in different laboratories. The potassium-induced increase in swelling (IV B, 2b) has thus been observed to develop in the interval 14-21 days in one rat population (Schousboe, 1972) but between 20 and 30 days in another population (Franck, 1970).

Also during phylogenesis great alterations occur in the distribution between nerve cells and glial cells. The relative amount of the neuropil seems thus to increase with an increasing brain size (Fig. V, 3) leading to a decreased neuronal density (e.g. Nissl, 1898; Economo, 1926; Tower & Elliott, 1952; Tower, 1954; Friede, 1954; Hawkins & Olszewski, 1957; Haug, 1960; Friede & Van Houten, 1962; Altman, 1967, cf. however also Nurnberger & Gordon, 1957). The interesting hypothesis has been forwarded by Roitbak (1970) that it should be possible to form conditioned reflexes only in species with a relatively high degree of differentiation of the glial cells.

Phylogenetically less developed species often contain special forms of glia cells

(Johnston & Roots, 1972), e.g. the leech packet glia cell. This cell is well suited for neuro-physiological investigation on account of its large size and has been extensively used in several important studies on glial function by the Kuffler group (see, e.g. Kuffler & Nicholls, 1966; Kuffler, 1967, cf. also IV A, 3d and IV B, 4a). As can be seen from Fig. I, 7, the packet glial cell differs, however, morphologically very much from the astrocytes and oligodendrocytes found in mammalian brain. Since the leech central nervous system in addition contains a centrally located neuropil (Coggeshall & Fawcett, 1964) in which all the synaptic contacts occur (Johnston & Roots, 1972), great caution should probably be taken in attributing a too general validity to results obtained with this and other invertebrate preparations.

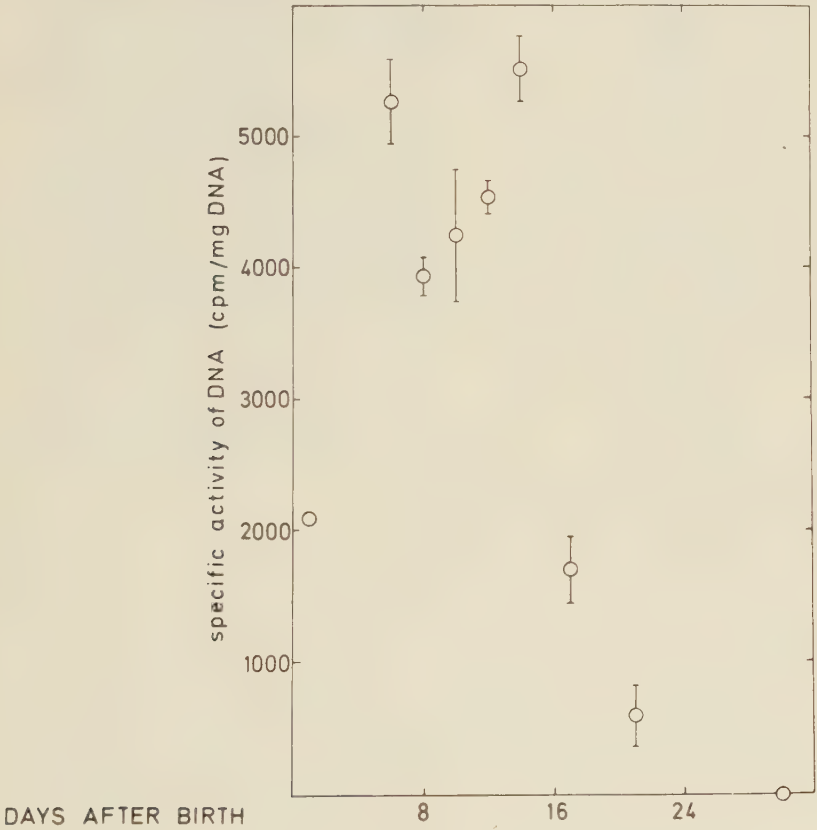


Fig. I, 5. Rate of DNA synthesis measured as in vivo incorporation of thymidine-2- [¹⁴C] into DNA (cpm/mg DNA) of the rat brain cortex 12 hrs after intraperitoneal injection of [¹⁴C] thymidine to rats between one day of age and maturity. The brain region used was the superficial 0.5 mm of the outer convexity of each hemisphere, i. e. corresponding to the first brain-cortex slice cut from the lateral convexity.

From Schousboe, 1972.

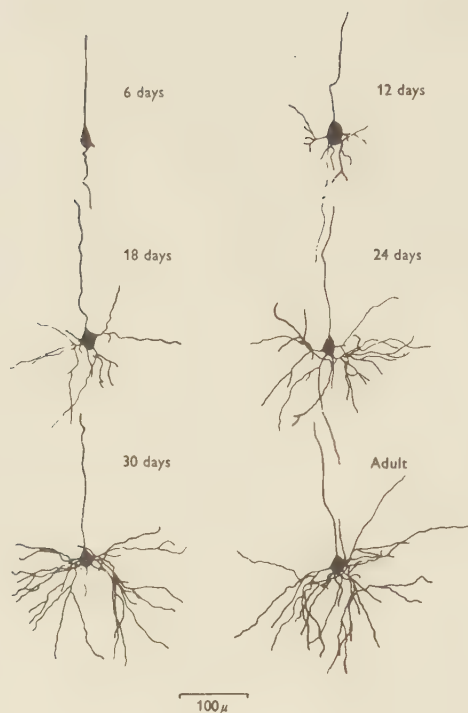


Fig. 1, 6. Characteristic changes in appearance of pyramidal cells in the rat brain cortex (lamina ganglionaris) during ontogenesis.

From Eayrs & Goodhead, 1959.

5. Compartmentation

The presence of metabolic compartmentation in brain tissue in vivo or in vitro (Berl, Lajtha & Waelsch, 1961; Berl, Nicklas & Clark, 1968; cf. also V A, 2) is a further indication of cellular or subcellular heterogeneity. A great advantage of compartmentation studies is their use of histologically intact preparations but, on the other hand, compartmentation studies alone give no information about the cellular or subcellular localization. This is also the case with experiments involving resolution of curves describing ion fluxes (e. g. Franck, 1970) which have been used to obtain a differentiation of various cellular compartments characterized by different membrane permeability for ions (cf. IV A, 3e).

6. Subcellular fractionation

Brain tissue may in analogy with tissue from most other organs be subfractionated into various cellular elements (Whittaker, 1959; Gray & Whittaker, 1960, 1962; De Robertis, De Iraldi, De Lorez Arnaiz & Salganicoff, 1962; De Robertis, 1967; Whittaker & Sheridan, 1965). The great complexity of the brain necessitates special precautions to prevent contamination of the individual fractions (e.g. Whittaker, 1968; Clark & Nicklas, 1970).

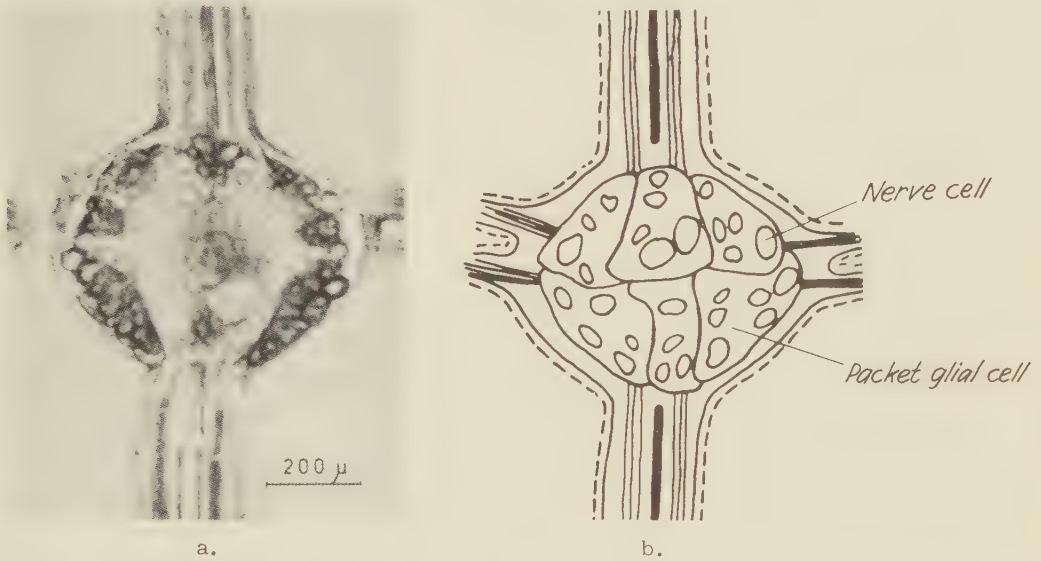


Fig. 1, 7a: Photograph of a living leech ganglion seen in isolation with transmitted illumination during experimentation. Dorsal view, showing the centrally located neuropil (pale area) which receives processes from the neurons in the "packets" and from other regions of the body. Individual neurons are easily distinguished. The numerous nerve fibers in the connectives and roots appear as dark strands. Glial cells are transparent and surround the neurons. Each ganglion contains about 350 unipolar nerve cells which are grouped in six separate clusters or "packets". All the nerve cells and their initial axonal processes within one packet are embedded in a single large glia cell.

b: Diagram of a portion of the leech central nervous system. One ganglion is shown from the ventral side with its six separate clusters (packets) of nerve cells.

From Kuffler & Nicholls, 1966.

Oxygen Consumption

A. RATE OF OXYGEN UPTAKE BY BRAIN

1. Interspecies variation

The rate of oxygen consumption by brain (and other tissues) decreases with an increasing size of the animal as shown in Fig. II, 1 (Quastel & Wheatley, 1932; Elliott & Henderson, 1948; Krebs, 1950; Elliott, 1952, 1957; Davies, 1961; cf., however, also Locker & Kaps, 1960). This correlation is almost, but not totally similar to that observed for neuronal density which decreases with an increasing brain size (I B, 4). Accordingly, e.g. the ox brain seems to have a higher neuronal density (Fig. V, 3) but a lower respiratory intensity (Fig. II, 1) than human brain.

2. Topographical variation

Also the structure from which the tissue has been removed is important for the respiratory intensity. In table II, 1 an attempt has been made to introduce a correction for the species difference (cf. Fig. II, 1) by recalculating all values to their supposed magnitude in human tissue as indicated in the legend of the figure. Slices from cerebral and cerebellar cortex respire at approximately equal intensity. In basal ganglia the respiratory rate is the same or somewhat lower (Dixon & Meyer, 1936; Bollard & McIlwain, 1957; Hertz & Clausen, 1963; Ridge, 1967; Weiss, Hertz & Goodman, 1972). The rate of oxygen uptake is still lower in the brain stem (Bollard & McIlwain, 1957; Hertz & Clausen, 1963; Ridge, 1967), and shows a decline from the rostral part to the caudal part (Hertz & Clausen, 1963). This trend towards a decrease continues to the spinal cord and peripheral nerve, which respire at an intensity of only 10-20 per cent of that in the cortex (Holmes, 1932;

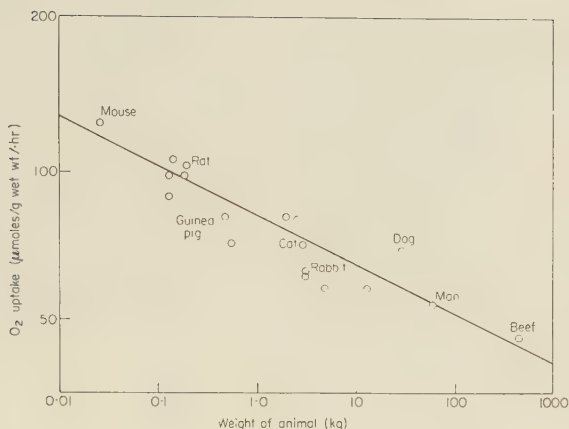


Fig. II, 1. Correlation between rate of oxygen uptake in brain cortex slices and body weight in different species. Both axes are drawn on logarithmic scales and each point represents an individual animal (except in the case of mice for which pooled slices from several animals were used).

From Elliott & Henderson (1948).

Chang, Shaffer & Gerard, 1935; Chang & Tai, 1936; Hertz & Clausen, 1963), whereas peripheral ganglia show a relatively high rate of oxygen uptake (Larrabee, 1958; Nagata, 1969; cf. however also Holmes, 1932; Dixon & Meyer, 1936).

The rate of oxygen uptake by white matter from the centrum semiovale of the calf brain or the corpus callosum of the rabbit brain is low (Hertz & Clausen, 1963; Ridge, 1967), but subependymal white matter from the guinea pig brain and human subcortical white matter show respiratory rates which are about half of those obtained with cortical slices (Bollard & McIlwain, 1957; Kurokawa, 1960; Hertz & Clausen, 1963).

Retina which ontogenetically is a part of the central nervous system shows a rather high rate of oxygen uptake (Cohen & Noell, 1960).

In view of the great topographical variation, respiration by a cortical brain slice can only be compared to that of the total brain in vivo (or in vitro) or to that of a whole-brain homogenate when the fractions of white and grey matter in the latter preparations are known. Roughly, the rate of oxygen consumption in the total rat brain (per unit weight) has been estimated as corresponding to about 80 per cent of that in cortical brain slices (Elliott, 1952), and almost half of the human brain is made up by the cortex (Sholl, 1956).

Table II, 1. Rates of oxygen uptake in different structures.

Structure	Animal species	Resting rate of oxygen uptake (μ moles/g fresh wt/hr)	Stimulated rate of oxygen uptake (μ moles/g fresh wt/hr)
Cerebral cortex	Man (McIlwain, 1953a)	41-51	81*
	Rat (Hertz & Clausen, 1963)	50	79
	Guinea-pig (Bollard & McIlwain, 1957)	40	70*
	Rabbit (Ridge, 1967)+	47	86
	Ox (Hertz & Clausen, 1963)	54	85
	Average	47	80
Cerebellar cortex	Rabbit (Ridge, 1967)+	53	93
	Ox (Hertz & Clausen, 1963)	55	100
	Average	54	97
Caudate nucleus	Rabbit (Ridge, 1967)+	41	69
	Ox (Hertz & Clausen, 1963)	46	97
	Average	44	83
Diencephalon	Guinea-pig (Bollard & McIlwain, 1957)	38	
	Rabbit (Ridge, 1967)+	29	43
	Average	34	
Medulla oblongata	Guinea-pig (Bollard & McIlwain, 1957)	22	36*
	Rabbit (Ridge, 1967)+	17	21
	Ox (Hertz & Clausen, 1963)	14	13
	Average	18	23
Spinal cord (total transversal sections) (grey matter)	Ox (Hertz & Clausen, 1963)	9	9
	Ox (Hertz & Clausen, 1963)	29	26
Cerebral white matter (central parts)	Rabbit (Ridge, 1967)+	11	10
	Ox (Hertz & Clausen, 1963)	10	10
	Average	11	10
(subcortical)	Man (Bollard & McIlwain, 1957)	25	
	Guinea-pig (Bollard & McIlwain, 1957)	20	35*
	Guinea-pig (Kurokawa, 1960)	22	38
	Guinea-pig (Kurokawa, 1960)	22	40*
	Rabbit (Bollard & McIlwain, 1957)	11	

Rates of oxygen uptake by different brain tissue structures have been measured in various species. All respiratory rates here have therefore been recalculated so as to fit human tissue. This was done by multiplying the observed rates of oxygen uptake in rat, guinea-pig, rabbit and ox tissue by 0.55, 0.70, 0.80 and 1.25, respectively (cf. Fig. II, 1).

The first column shows the "resting" rates of oxygen uptake, whereas the second column shows the respiratory rates following the addition of excess (about 50 mM) potassium or during the application of electrical pulses. The experiments in which pulses were applied are indicated by *.

+ All these values (from Ridge 1967) have been multiplied by 0.5 since they differ from the rest by a factor of this magnitude.

(From Hertz, 1969.)

3. Variation between different modes of preparation

a. Intact brain: The mean oxygen consumption in normal human adults can be calculated to 3.6 ml/100 g per min (Kety, 1957), which equals 95 μ moles/g wet wt. per hr. (cf. table II, 2). Mental activity does not affect the oxygen consumption in the total brain, but seems to increase the metabolic activity in certain regions (cf. Risberg & Ingvar, 1968); a decrease of the metabolism to about 60 per cent is found in coma or under anaesthesia (Kety, 1957), and an increase occurs during convulsions (Schmidt, Kety & Pennes, 1945). Approximately identical results have been obtained in the isolated and perfused cat brain (cf. table II, 2) by Geiger and Magnes (1947).

b. Slices: Human brain-cortex slices respire at a rate of 40-50 μ moles/g fresh wt. per hr. during incubation in a "balanced" medium (Elliott & Henderson, 1948; McIlwain 1953a). This may correspond to a whole-brain respiration of 32-40 μ moles/g wet wt. per hr. (II A, 2), i.e. less than half of the respiratory intensity recorded in vivo (cf. table II, 2). Exposure to e.g. high concentrations of potassium or electrical pulses may, however, lead to an increase of almost 100 per cent in the respiratory rate by brain-cortex slices (cf. II B, 2a) or the locally perfused cerebral cortex in vivo (Grenell, 1959). The magnitude of the stimulated respiration is accordingly much more similar to that of the functioning brain (cf. Quastel & Quastel, 1961; McIlwain, 1966, p. 55).

c. Isolated cells: The oxygen uptake by large neurons (table II, 3) obtained by microdissection amounts to 50-100 $\times 10^{-5}$ μ l/hr. per cell (Epstein & O'Connor, 1965; Hydén & Lange, 1965; Hertz, 1966). This value is in relatively good agreement with a mean nerve cell respiration of about 2-14 $\times 10^{-5}$ μ l/hr. per cell calculated on the basis of respiratory rates in fractions from white and grey matter (Heller & Elliott, 1955; Elliott & Heller, 1957; Korey & Orchen, 1959 - cf. however also II C), and with a respiratory rate of 0.7 $\times 10^{-5}$ μ l/hr. per cell in small cultivated cells (Dittmann et al. 1973c). Similarly, the rate of oxygen uptake per glia cell (table II, 3) has been both measured and calculated to 0.5-1 $\times 10^{-5}$ μ l/hr. per cell (Heller & Elliott, 1955; Korey & Orchen, 1959; Hydén & Lange, 1965; Hertz, 1966; Dittmann et al., 1973c). The microdissected neuroglial samples obtained by Epstein & O'Connor

Table II, 2. Corrected rates of oxygen uptake by different brain preparations

Preparation	Animal species		Rate of oxygen uptake μ moles/g wet wt./hr.
<u>in vivo</u> , normal conditions	man	1)	95
<u>in vivo</u> , coma	man	1)	50
<u>in vivo</u> , convulsions	monkey	2)	140
perfused brain, normal conditions	cat	3)	90
perfused brain, coma	cat	3)	50
perfused brain, convulsions	cat	3)	180
brain slices, resting conditions	man	4)	45
brain slices, electrical stimulation	man	4)	90
brain slices, excess potassium	rat	5)	65
homogenates, resting conditions	rat	6)	40
homogenates, excess potassium	rat	6)	35
isolated neurons, resting conditions	cat	7)	150
isolated neurons, excess potassium	cat	7)	140
isolated glia cells, resting conditions	rat	7)	25
isolated glia cells, excess potassium	rat	7)	50
isolated neurons plus glia cells in a ratio similar to that in brain cortex	rat + cat	7)	30
do., excess potassium		7)	55

The rates of oxygen uptake by the different structures were measured in different species (indicated in the table), and have been recalculated to the supposed values in human tissue. This was done by multiplying the observed rates of oxygen uptake in rat, cat and monkey tissue by respectively 0.55, 0.70 and 0.80 (cf. Fig. II. I).

The average respiratory intensity in the rat brain corresponds to about 80 per cent of that in a rat brain-cortex slice (cf. II A, 2). On the assumption that this correlation also is valid in other species and after homogenization or microdissection, all results obtained with slices, homogenates or isolated cells were furthermore multiplied by 0.80 to give the corresponding respiratory rates by the whole brain.

As a result of the corrections only approximated values are presented.

No attempt has been made to indicate the scatter of the figures given in the literature, but the values shown are supposed to be representative. In general the deviations from these values are small and may be estimated e. g. from table II, 1 and VI, 1.

Results originate from:

1) Kety, 1957; 2) Schmidt *et al.*, 1945; 3) Geiger & Magnes, 1947; 4) McIlwain, 1953c; 5) Hertz & Schou, 1962; 6) Elliott & Libet, 1942 (average values from their table 2); 7) Hertz, 1966.

Table II, 3.

Cell	Origin	Species	Method	O ₂ uptake per cell, $\mu\text{l} \times \text{hr}^{-1}$	O ₂ upt. per vol. $\mu\text{moles} \times \text{hr}^{-1}$ per g wet wt.	Authors
Neuron	cortex	cat	microdissection	101×10^{-5}	1080	Epstein & O'Connor, 1965
Neuron	brain stem	rabbit	microdissection	100×10^{-5}		Hydén & Lange, 1965
Neuron	cortex	cat	microdissection	50×10^{-5}	263	Hertz, 1966
Neuron	cortex	rat	gradient centri-fugation	3.0×10^{-5}	79	Rose, 1967
Neuron	cortex	rabbit	gradient centri-fugation		204	Haljamäe & Hamberger, 1971
Neuron	cortex	rat	gradient centri-fugation	0.15×10^{-5}	95	Hemminki & Holmila, 1971
Neuron	cortex	rat	gradient centri-fugation	0.11×10^{-5}	88	Hemminki & Holmila, 1971
Neuron	spinal ggl.	chick	microdissection	4×10^{-5}	1000	Hartman et al., 1970
Neuron	spinal ggl.	chick	cultivation	4.3×10^{-5}		Booher et al., 1971b
Neuron	cortex	chick	cultivation	0.66×10^{-5}	567	Dittmann et al. 1973c
Neuron	cortex	cat, dog	calculation	$6-9 \times 10^{-5}$		Heller & Elliott, 1955
Neuron	cortex	cat, dog	calculation	$2-3 \times 10^{-5}$		Elliott & Heller, 1957
Neuron	cortex	lamb	calculation	14×10^{-5}		Korey & Orchen, 1959
Glia cell	cortex	rat	microdissection	0		Epstein & O'Connor, 1965
Glia cell	brain stem	rabbit	microdissection	$0-10 \times 10^{-5}$		Hydén & Lange, 1965
Glia cell	cortex	rat	microdissection	$0.5-1 \times 10^{-5}$	40-80	Hertz, 1966
Glia cell	cortex	rat	gradient centri-fugation		76	Rose, 1967
Glia cell	cortex	rabbit	gradient centri-fugation		187	Haljamäe & Hamberger, 1971
Glia cell	cortex	rat	gradient centri-fugation	0.11×10^{-5}	71	Hemminki & Holmila, 1971
Glia cell	cortex	rat	gradient centri-fugation	0.04×10^{-5}	40	Hemminki & Holmila, 1971
Astrocyte	cortex	rat	cultivation	0.75×10^{-5}	130	Dittmann et al., 1973c
Glia cell	c. callosum	cat, dog	slices	$0.5-0.6 \times 10^{-5}$		Heller & Elliott, 1955
Glia cell	c. callosum	lamb	homogenate	1.2×10^{-5}		Korey & Orchen, 1959

Table II, 3. Rates of oxygen uptake (per cell and per volume) given in the literature for neurons and glial cells. The values were obtained by microdissection, cultivation, gradient centrifugation or calculation (based upon cell counts and respiratory rates in slices or homogenates of corpus callosum). The oxygen uptake rates per cell obtained by gradient centrifugation have been recalculated from the dry wt. or the content of protein per cell given in the original papers, and the corresponding respiratory rates per volume have been calculated on the assumption that solids constitute 20 % and protein 10 % of the cell volume.

Based upon the volume data presented in some of the papers the neurons may be classified as large (microdissected samples), medium sized (spinal ganglion) or small (present results). The calculated results and probably also the values for cells obtained by centrifugation (cf. Rose, 1967) refer to cells of average size. From Dittmann et al., 1973c.

(1965) showed practically no oxygen uptake at all, but a long period of preincubation (2 hrs) had been employed, and the respiration by isolated glia clumps declines rapidly (Hertz, 1966). Cells obtained by the different gradient centrifugation techniques show a variable and often low rate of oxygen uptake (table II, 3; cf. also Rose & Sinha, 1969).

Expressed per unit volume (or weight) somewhat less consistency is found. In neuronal- and glial-enriched fractions obtained by differential centrifugation, the rate of oxygen uptake has been found to 70-200 μ moles/g fresh wt. per hr. (table II, 3). Measurements on microdissected (Hertz, 1966) or cultivated (Dittmann et al., 1973c) cells or calculation (Korey & Orchen, 1959) give comparable values in the case of glia cells (table II, 3), whereas the respiration of microdissected and of cultivated neurons has been found to be higher, i. e. between 260 (Hertz, 1966) and 1080 (Epstein & O'Connor, 1965) μ moles/g fresh wt. per hr. (cf. however also the low values for young embryonic neurons observed by Dittmann et al., 1973b). Reactive cortical glia cells (Ruscak, Ruscaková & Koniková, 1967) and astrocytoma cells (Victor & Wolf, 1937; Heller & Elliott, 1955; Allen, 1957, 1972) show a much lower respiratory activity but it should be kept in mind that rapidly proliferating cells may have a decreased respiration (Nissen, Ciesielski-Treska, Hertz & Mandel, 1972, 1973).

Nerve-ending particles prepared by differential and density gradient centrifugation respire at a rate of 50-60 μ moles/100 mg protein per hr. (Bradford, 1967; 1969; De Belleruche & Bradford, 1972) which probably corresponds to about 50-60 μ moles/g wet wt. per hr. (cf. also Marchbanks & Whittaker, 1967).

On the assumption that the nerve cell perikarya account for 5 % of the total volume (I B, 1), summation of the respiratory rate by the nerve cell perikarya and that by the microdissected neuropil clumps yields a rate of oxygen uptake which is reasonably close to that observed with brain slices (Hertz, 1966 - cf. table II, 2). A similar calculation based upon the values obtained with cultivated cells (table II, 3) indicates that the nerve cell bodies and astrocytes in a brain-cortex slice containing 5 % nerve cell bodies (I B, 1) respiring at a rate of 500 μ moles/g

wet wt. per hr. (cf. above) and 30 % astrocytes (I B, 1) respiring at a rate of 100 μ moles/g per hr. (cf. above) can be expected to show an oxygen consumption rate of 25 (5 % of 500) plus 30 (30 % of 100), i.e. 55 μ moles/(g wet wt. of the total preparation) per hr. The extracellular space (about 25 % according to table IV, 1) can not be expected to show any respiratory activity, and the remaining part of the preparation (neuronal processes, nerve-ending particles, glia cells apart from astrocytes) i.e. 40 (100-(5+30+25)) % of the volume must account for the remaining respiratory activity which thus amounts to 90 (rate of oxygen uptake by rat brain-cortex slice) minus 55, i.e. 35 μ moles/(g wet wt. of the total preparation) per hr. The average rate of oxygen uptake in the neuronal processes, the nerve-ending particles and the non-astrocytic glia cells should accordingly be 35 μ moles/(g wet wt. of the total preparation) per hr. or $\frac{35 \times 100}{40}$, i.e. 85-90 μ moles/(g wet wt. of the component in question) per hr. This value, which probably is encumbered with a considerable uncertainty is rather close to the about 60 μ moles/g fresh wt. per hr. observed in nerve-ending particles which may indicate that the neuronal processes do not have a specially high rate of oxygen uptake (cf. II B, 1b).

d. Homogenates and cell suspensions: Homogenized preparations show an initial rate of oxygen uptake which generally is relatively close to that in a corresponding slice (Elliott & Libet, 1942; Elliott & Henderson, 1948 - cf. also table II, 2) but the respiratory intensity often declines rapidly (cf. Fig. II, 2 and II B, 1c). Cell suspensions obtained by passing brain cortex cubes through nylon sieves (Hemminki & Huttunen, 1970) show, in contrast, a well maintained rate of oxygen uptake (Kovaru - Mlejnková, 1971).

B. ION EFFECTS ON OXYGEN CONSUMPTION

1. Maintenance of respiration

a. Slices: Under optimum conditions the oxygen consumption by brain slices may be extremely well maintained (see, e.g. Fuhrman & Field, 1948). This requires, among other things, an appropriate ionic composition of the incubation medium.

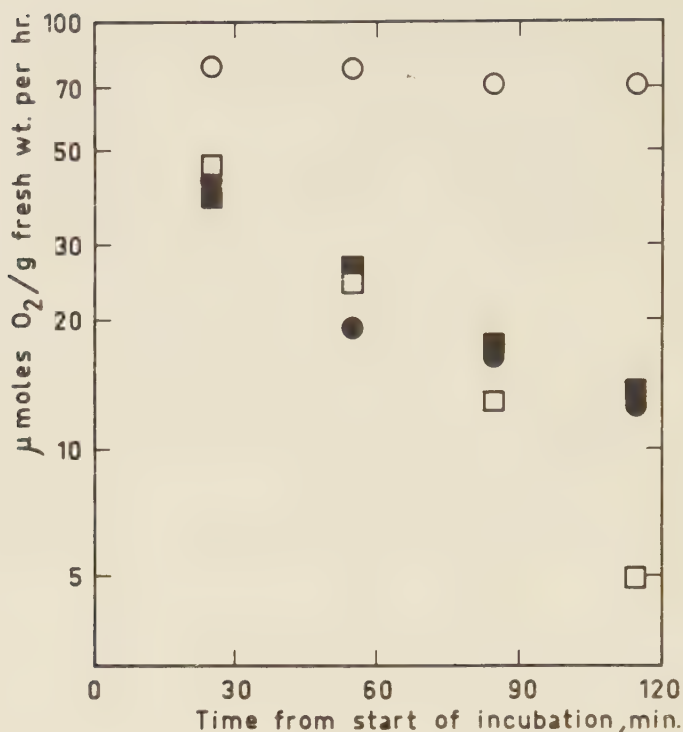


Fig. II, 2. Rates of oxygen uptake by rat brain-cortex slices (o and ●) and by homogenates prepared from corresponding slices (□ and ■) during incubation in "balanced" media (o and □) or in media in which all sodium chloride had been replaced by an isosmotic amount of sucrose (● and ■).

The rates of oxygen uptake are plotted on a logarithmic scale as a function of the time (min.) from the start of the incubation.

Results are means of 3 unpublished experiments by L. Hertz.

Sodium: During incubation in media where sodium has been replaced with sucrose, the respiratory rate of brain cortex slices shows a rapid exponential decline, until a resistant fraction of about 10 per cent of the initial rate of oxygen uptake is reached (Gore & McIlwain, 1952, Hertz & Clausen, 1963; Hertz, 1966). This respiration continues almost unaffected for hours. The respiratory decline is irreversible. Later addition of sodium may prevent a further decrease but does not cause any return to the previous level (Hertz & Schou, 1962). This seems to indicate, that the rate of oxygen uptake is no simple function of the sodium concentration in the tissue (cf. however also Whittam, 1962b) and is in keeping with the absence of morphological recov-

ery after incubation in sodium-deficient media (Ibata et al., 1971, cf. also IV B, 3b and IV B, 4d).

Choline is the only ion which is able to replace sodium in maintaining the oxygen consumption of brain cortex slices at an almost normal level (Takagaki & Tsukada, 1957; Hertz & Schou, 1962; Abadom & Scholefield, 1962b). Neither K^+ , Cs^+ , Rb^+ or Li^+ possess this ability, and the respiration declines especially fast in a medium in which Na^+ has been replaced by Li^+ (Hertz & Schou, 1962). The rate of oxygen uptake by spinal cord, white matter and peripheral nerve is, in contrast, well-maintained in a medium in which sodium has been replaced with sucrose (Hertz & Clausen, 1963), though its level seems to be somewhat decreased (Chang et al., 1935). Kidney slices do also not require sodium to maintain their rate of oxygen uptake, whereas the respiration of rat hemidiaphragm decreases rapidly during incubation in a medium in which all sodium has been replaced with sucrose (Hertz & Clausen, 1963).

Potassium: Also potassium ions are required in the medium to maintain respiration, but only in a concentration of 1-2 mM. This low K^+ concentration may under ordinary experimental conditions be obtained in potassium-free media by leakage from the tissue, and it has been reported that potassium is neither essential to maintain respiration (Takagaki & Tsukada, 1957) nor required in the electrical stimulation of brain slices (Gore & McIlwain, 1952, cf. however also Cummins & McIlwain, 1961 and II B, 2a). Studies on slices which after an initial potassium loss to a potassium-free medium were transferred to fresh, potassium-free media, have, however, revealed a rapid respiratory decline (Fig. II, 3) from an initially slightly increased value.

Potassium concentrations above 20 mM interfere with the maintenance of respiration (Hertz & Schou, 1962; Mase, Takahashi & Ogata, 1962; Machiyama, Balazs, Hammond, Julian & Richter, 1970). A similar effect is evoked by NH_4^+ , Cs^+ , Rb^+ , Li^+ and choline. The higher the concentration of potassium (or other stimulating ion) is, the faster the respiratory decline occurs (Fig. II, 4), but the increase in the rate of respiratory decline may be more or less effectively counteracted by a raise of the sodium concentration in the medium (Hertz & Schou, 1962).

Electrical stimulation: Also application of electrical pulses may lead to an increase in the rate of respiratory decline (e. g. Wallgren & Kulonen, 1960; Jones & Banks, 1970a) but this is not always evident (e. g. McIlwain, 1951a). Under sub-optimum conditions the decline may, however, involve that the "stimulated" respiration becomes lower than the unstimulated rate of oxygen uptake (Cummins & McIlwain, 1961).

Calcium and magnesium: The presence of calcium and possibly also of magnesium in concentrations of 1-2 mM are required to maintain the respiration (Dickens & Greville, 1935).

b. Isolated cells: The neuroglia lumps isolated by microdissection show a rapidly declining rate of oxygen uptake even during incubation in a "balanced" medium. Addition of high concentrations of potassium or replacement of all sodium chloride with sucrose leads to a further increase in the rate of respiratory decline (Hertz, 1966). Incubation of brain slices in sodium-deficient media affects the morphology of the glial cells more than that of the neurons (Ibata et al., 1971).

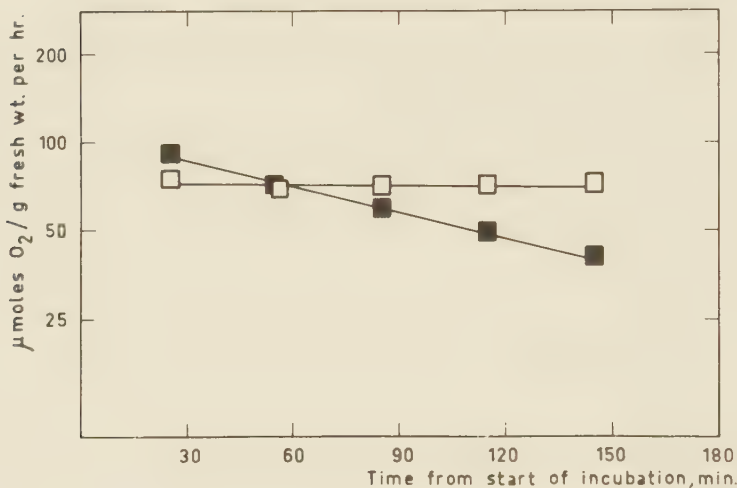


Fig. II, 3. Rates of oxygen uptake by rat brain-cortex slices which after an initial loss of their potassium content in a cold, potassium-free medium (30 min.) were transferred to fresh media containing either 0 (■) or 2.5 (□) mM potassium.

The rates of oxygen uptake are plotted on a logarithmic scale as a function of the time (min.) from the start of the incubation following the transfer.

Results are means of 3 and 7 unpublished experiments by L. Hertz. S.E.M. do not extend beyond the symbols.

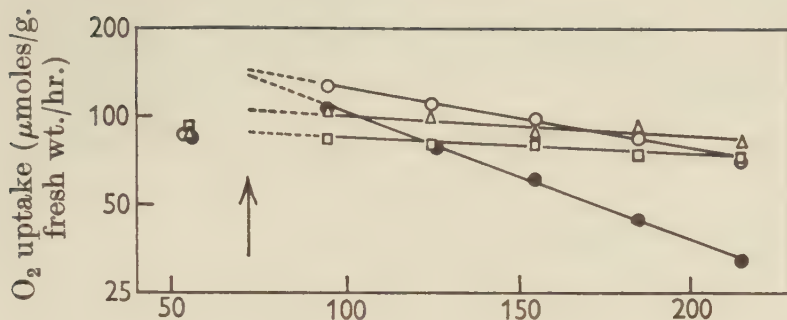


Fig. II, 4. Rates of oxygen uptake by rat brain-cortex slices before and after the addition (arrow) of 16 mM-(□), 32 mM-(△), 48 mM-(○) or 384 mM-(●) potassium chloride to a medium containing 125 mM sodium chloride. Extrapolated oxygen-uptake rates are indicated by broken lines.

Results are means of 2-3 experiments. From Hertz & Schou, 1962.

The nerve cell bodies obtained by microdissection maintain a high rate of oxygen uptake for hours (Epstein & O'Connor, 1965; Hertz, 1966). This is also the case during incubation in potassium-rich (Aleksidze & Blomstrand, 1969) or sodium-free media (Hertz, 1966). The absolute level of the respiratory rate may however be somewhat reduced in the absence of sodium (Hertz, 1966). - On the basis of these findings it has been suggested (Hertz, 1966) that only the part of the oxygen uptake in a brain slice that continues for hours in a sodium-free medium (i. e. about 20 per cent of the resting respiration - cf. IIB, 1a) should occur in the nerve cell bodies, whereas the remaining - larger - fraction of the oxygen uptake should occur in the neuropil, i. e. probably mainly in neuroglia (cf. I B, 1). The latter concept is supported by the observation that two thirds of the oxidative metabolism in thalamus (measured as cytochrome oxidase activity) remains intact after degeneration of the neurons by decortication (Howe & Mellors, 1945) and by the rather high respiratory activity of glia cells (II A, 3c). It is however, at variance with calculations made on the basis of counts of glia cells and nerve cells and the respiratory rates per cell (cf. II A, 1c), which indicate that about 3/4 of the oxygen uptake should occur in the nerve cells and only about 1/4 in the glia cells (Heller & Elliott, 1955; Elliott & Heller, 1957; Korey & Orchen, 1959; Tower, 1960; cf. also Giacobini, 1964). It is also not in agreement with the conclusion (based upon measurements of enzyme activities in brain cortex areas, which are rich in dendrites, but also contain glia cells) that brain metabolism mainly should occur in the dendrites (Lowry, Roberts, Leiner, Wu, Farr & Albers, 1954 - cf. however also Strominger & Lowry, 1955 and II A, 3c).

c. Homogenates: In thoroughly dispersed homogenates the respiration is less well maintained than in slices (Fig. II, 2; Fig. 3 in Elliott & Libet, 1942; Heald, 1960, p. 75; cf. also Bradford, 1969)), and the ability of the sodium ion to maintain a steady respiration may be almost completely lost (cf. however also Whittam, 1962b).

2. Stimulation of respiration

a. Slices: The rate of oxygen uptake by brain slices may be increased by exposure to certain "stimulating" ions or to electrical pulses.

Potassium and related monovalent cations: The best known "stimulating" ion is potassium (e.g. Ashford & Dixon, 1935; Dickens & Greville, 1935). A potassium concentration of about 20 mM is required to exert a respiratory stimulation in brain-cortex slices (Hertz & Schou, 1962; Huttunen, 1969) and the presence of approximately 50 mM may lead to almost a doubling of the rate of oxygen consumption (Fig. II, 5). Still higher concentrations have a somewhat smaller effect but this may be due to an increased rate of respiratory decline. The maximum increase in oxygen uptake seems to occur very rapidly after the increase in the potassium concentration (Fig. II, 6), i.e. before any pronounced changes have occurred in the tissue concentrations of monovalent cations (cf. IV B, 3ab; Lund-Andersen & Hertz, 1970). Previous exposure to media deprived of potassium prevents the response when the high concentrations of potassium are subsequently added (Dickens & Greville, 1935). The stimulating group of ions also comprises ammonium (Weil-Malherbe, 1938; Gore & McIlwain, 1952), cesium, rubidium (Dickens & Greville, 1935) lithium (Canzanelli, Rogers & Rapport, 1942; Rybová, 1959) and choline (Hertz & Schou, 1962). The maximum rate of oxygen uptake caused by all the stimulating ions is identical (with the possible exception of choline), but the concentration required to obtain maximum (or half-maximum) effects vary (Fig. II, 5) so that ammonium exerts the strongest and choline the weakest action (Hertz & Schou, 1962).

The potassium-induced stimulation of oxygen uptake is absent in brain cortex slices from rats younger than 2 weeks (Himwich, Bernstein, Fazekas, Herrlich & Rich, 1942) and also the responsiveness to electrical stimulation is decreased at this age (Greengard & McIlwain, 1955b; McIlwain, 1956b).

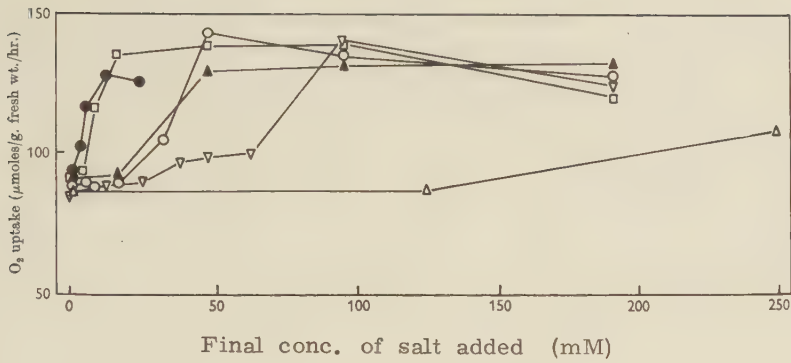


Fig. II, 5. Rates of oxygen uptake by rat brain-cortex slices immediately after the addition of ammonium chloride (●), cesium chloride (□), rubidium chloride (▲), potassium chloride (○), lithium chloride (▽) or choline chloride (△) to media containing 125 mM sodium chloride. Results are means of 2-4 experiments. From Hertz & Schou, 1962.

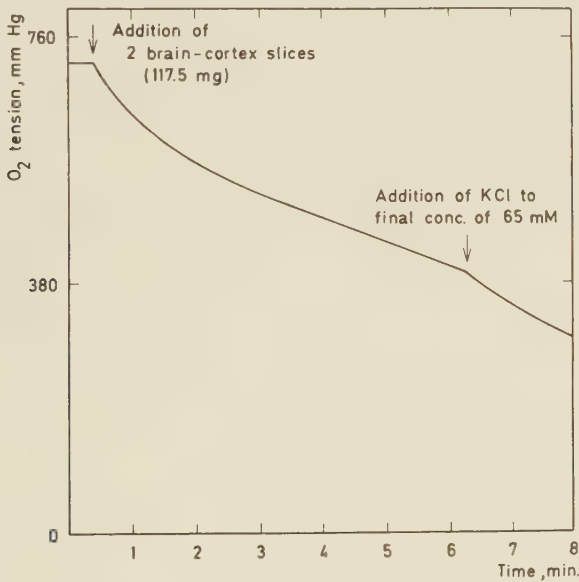


Fig. II, 6. Tracing of typical record obtained with the oxygen electrode in a 3.0 ml chamber and showing the rate of oxygen uptake (slope of the curve) for rat brain-cortex slices in a bicarbonate-buffered, initially "balanced" medium. Addition of excess potassium (arrow) is seen to evoke an abrupt increase in the respiratory rate. Unpublished experiment by G. B. Weiss, F. Goodman & L. Hertz.

Other stimulating procedures: Exposure to a calcium-deficient medium (Dickens & Greville, 1935; Kratzing, 1953; Quastel & Quastel, 1961, Bourke & Tower, 1966b), application of electrical pulses (McIlwain, 1951a; McIlwain & Joanny, 1963) or addition of e. g. 0.01-0.05 mM dinitrophenol (e. g. McIlwain & Gore, 1952; Kratzing & Narayanaswami, 1953; Abadom & Scholefield, 1962a; Mase *et al.*, 1962; Gonda & Quastel, 1963; Okamoto & Quastel, 1970a) or of "labilizers" (Shanes, 1958a) like 0.001-0.01 mM protoveratrine (Wollenberger, 1955a; Quastel, 1960; Yoshida, Fujisawa & Kajikawa, 1963) cause an increase of the oxygen uptake, and the maximum rate of oxygen uptake is similar to that observed after addition of potassium (e. g. McIlwain, 1951b). It is not possible to exceed this respiratory rate, e. g. by simultaneous exposure to stimulating ions and electrical pulses, but an additive (or even a potentiated) effect of submaximum stimuli may be seen (Wollenberger, 1955b). The electrical stimulation may be maintained during more than one hour, and the rate of oxygen consumption remains increased during a short period (i. e. generally less than one minute) after the termination of the stimulation (McIlwain, 1954). The presence of a minimum amount of potassium is essential both for the electrical stimulation (Cummins & McIlwain, 1961) and for the stimulation evoked by dinitrophenol (Bilodeau & Elliott, 1963) or veratrine (Wollenberger, 1955a; Bilodeau & Elliott, 1963). Also addition of L-glutamate causes an increase in the oxygen consumption of brain slices (Krebs, 1935; Weil-Malherbe, 1936; 1960; Lipsett & Crescitelli, 1950; McIlwain, 1953a; Rossiter, 1955; Tsukada, Takagaki & Hirano, 1958; Takagaki, Hirano & Nagata, 1959; Gonda & Quastel, 1963 - cf. also table VI, 1 and III B, 6; IV B, 1; IV B, 2c; IV B, 3a; IV B, 4c), whereas the D-isomer may be less effective (Takagaki *et al.*, 1959 - cf. however also Takagaki, 1971). 1-10 mM GABA causes a corresponding increase, but lower concentrations are without effect on the respiratory rate (Balázs, Machiyama, Hammond, Julian & Richter, 1970). Acetylcholine may give rise to a small increase in the rate of oxygen consumption (Yoshida & Quastel, 1962; cf. however also McIlwain, 1951b; Wallgren, 1961; Nakazawa & Quastel, 1968b), and it has been suggested that only cholinceptive neurons should be affected (Brossard & Quastel, 1963).

Calcium and magnesium: A substantial increase in the calcium concentration from the 1-2 mM present in a "balanced" medium leads to a decrease of the oxygen uptake in brain slices (Charnock, 1963) but to an increase in muscle (Simon, Muller & Satchell, 1962). Minor augmentations (i. e. to 5-8 mM) are without effect on both the resting and the stimulated respiration in brain, (Gore & McIlwain, 1952), and a reduction leads to an increased rate of oxygen uptake (cf. above). High concentrations (i. e. about 10 mM) of magnesium may counteract the stimulation after addition of excess potassium (Hertz & Schou, 1962 - cf. also Nakazawa & Quastel, 1968a).

Sodium: The potassium-induced and the electrical stimulation of oxygen consumption requires the presence of a certain concentration of sodium (e.g. Dickens & Greville, 1935; Canzanelli *et al.*, 1942; Gore & McIlwain, 1952; Hertz & Schou, 1962; Bachelard, Campbell & McIlwain, 1962; Tsukada & Takagaki, 1955; Kosawa & Naito, 1966; Nakazawa & Quastel, 1968a), whereas glutamate (Takagaki *et al.*, 1959) and dinitrophenol (Tsukada & Takagaki, 1955) have been observed to evoke a respiratory stimulation in the absence of sodium. Lithium is the only ion which is able to replace sodium (Hertz & Schou, 1962). This might indicate that the potassium ions exert their stimulatory effect at a potassium-sensitive site of an enzyme like the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ involved in active transport (VI B, 4c). An increased concentration of sodium exerts a competitive inhibition of both the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ (Whittam & Ager, 1962) and the potassium-induced stimulation of respiration. The latter effect has been described in some detail by Hertz & Schou (1962) but is also obvious from Fig. 3 in a paper by Elliott & Bilodeau (1962). - In muscle, the potassium-induced stimulation of oxygen uptake is, in contrast, independent of the presence of sodium (Novotny & Vyskocil, 1965; cf. also Hill & Howarth, 1957).

Augmentation of the sodium concentration above the level in a "balanced" medium does not lead to an increase in the rate of oxygen uptake by brain slices (Hertz & Clausen, 1963; Chan & Quastel, 1970), but stimulates respiration in muscle (Muller, 1962; Hertz & Clausen, 1963; Weinschelbaum de Jairala, 1964; Nissan, Aviram, Czaczkes, Ullmann & Ullmann, 1966).

Drug inhibition: Several drugs, e.g. barbiturates (McIlwain, 1953b; Ghosh & Quastel, 1954) have been observed to exert a pronounced inhibition of the stimulated respiration, but have also some effect on the resting oxygen uptake (Webb & Elliott, 1951; McIlwain, 1953b; Ghosh & Quastel, 1954; Weiss *et al.*, 1972). The inhibition sets in promptly after the addition of the drug (McIlwain, 1953b; Weiss *et al.*, 1972). The barbiturates inhibit the stimulation regardless whether it is evoked by application of electrical pulses (e.g. McIlwain, 1953b), high concentrations of stimulating ions (e.g. McIlwain, 1953b; Ghosh & Quastel, 1954), dinitrophenol (McIlwain, 1953b; Ghosh & Quastel, 1954), or labilizers (Quastel, 1959, 1960), but another group of drugs, including atropine (McIlwain, 1951b, 1953b; Wallgren, 1961), certain anticonvulsant hydantoin derivatives (Greengard & McIlwain, 1955a), tetrodotoxin (Nakazawa & Ogura, 1961; McIlwain, 1967; Chan & Quastel, 1967; Okamoto & Quastel, 1970b) and to some extent chlorpromazine (McIlwain, 1962; Quastel, 1965), procaine (Chan & Quastel, 1970) and cocaine (compare Geddes & Quastel, 1956, with McIlwain, 1951b; Chan & Quastel, 1970) inhibit the electrical stimulation with little or no effect on the stimulation exerted by high concentrations of potassium or dinitrophenol (McIlwain, 1951b; Greengard & McIlwain, 1955a; McIlwain & Greengard, 1957; Okamoto & Quastel, 1970b). Higher concentrations of at least some of these drugs

do, however, also affect the potassium induced stimulation (McIlwain, 1962; cf. also Weiss et al., 1972). The basic protein, protamine, inhibits both the potassium-induced and the electrical stimulation of the oxygen uptake, but is without effect on the respiratory increase evoked by dinitrophenol (McIlwain, 1959; McIlwain, Woodman & Cummins, 1961). Tetrodotoxin inhibits the stimulation evoked by calcium lack (Chan & Quastel, 1967), and fluoroacetate that caused by ammonium (Lahiri & Quastel, 1963) though these drugs in the same concentrations seem to be without effect on the potassium-induced stimulation of oxygen uptake.

Tissue specificity: Already Dickens and Greville (1935) noticed that the potassium-induced stimulation of oxygen uptake is not found with slices from the liver, the kidney or the testis. These results have later been confirmed and expanded, and it seems beyond doubt that neither kidney slices (Mudge, 1951; Taggart, Silverman & Trayner, 1953; Kleinzeller, 1961; Hertz & Clausen, 1963), liver slices (Elliott & Bilodeau, 1962; L. Hertz & T. Clausen, unpublished experiments), adrenal gland slices (Draskóczy, Burack & Weiner, 1963) or avian salt gland slices (Borut & Smith-Nielsen, 1963) show an increased rate of oxygen uptake when the potassium concentration in an otherwise normal medium is increased beyond a concentration of about 5 mM. In contrast, a slight decrease has often been observed (e.g. Hertz & Clausen, 1963). Peculiarly enough, addition of lithium to the salt gland slices was found to stimulate the oxygen consumption (Borut & Smith-Nielsen, 1963).

Similarly, application of electrical pulses or addition of "labilizers" or ouabain do not lead to an increased rate of oxygen uptake in slices of kidney (Kratzing, 1951; Wollenberger, 1955a; Joanny & Corriol, 1964), liver (Wollenberger, 1955a; Joanny & Corriol, 1964) or spleen (Wollenberger, 1955a). Application of dinitrophenol and related compounds does, in contrast, increase the metabolic rate by both liver, kidney (Ehrenfest & Ronzoni, 1933; McCord, 1934; Alwall, 1935) and adrenal gland (Draskóczy et al., 1963) slices.

Muscle seems to be the only tissue which resembles brain slices in showing a raised rate of oxygen uptake during incubation in media with increased concentrations of potassium, cesium, rubidium or lithium (Fenn, 1930; Hegnauer, Fenn & Cobb, 1934; Keynes & Maisel, 1954; Muller & Simon, 1960; Novotný, Vyskocil, Vycklický & Beránek, 1962; Hertz & Clausen, 1963; Freeman, Paddle, Gay & Naylor, 1965) or after application of electrical pulses (Kratzing, 1951; McIlwain, 1966, p. 55) or "labilizers" (Wollenberger, 1955a). This stimulation has, however, been less consistently reported than the similar phenomenon in brain slices (e.g. Zierler, 1956; Lee, Yu, Lee & Burstein, 1961; cf. also Mullaney, 1961), and a maxi-

mum response seems to occur with 20 mM potassium (Muller & Simon, 1960; Novotny et al., 1962).

Topographical specificity within the nervous system: Only tissue from structures that show a relatively high rate of oxygen uptake during incubation in "balanced" media, seems to react metabolically to excess potassium (cf. table II, 1). No stimulation is thus observed with mammalian peripheral nerve (Chang et al., 1935; Pietra, 1961; Tamarit & Gallego, 1962; Hertz & Clausen, 1963 - cf. however also Shanes & Hopkins, 1948) or with white matter from the central parts of the brain (Hertz & Clausen, 1963; Ridge, 1967), whereas subcortical white matter does show a distinct rise in oxygen uptake during exposure to excess potassium (Bollard & McIlwain, 1957; Kurokawa, 1960; Hertz & Clausen, 1963). The spinal cord of the calf shows no (Hertz & Clausen, 1963) and that of the frog or chicken little (Chang & Tai, 1936; H. Fosmark & L. Hertz, unpublished experiments) potassium-induced increase in the rate of oxygen uptake. A gradual change occurs in the brain stem, since slices from the lower part (i. e. medulla oblongata) of the calf brain do not react metabolically to excess potassium, whereas some response may be obtained in the middle part (pons), and tissue from the upper part (mesencephalon and colliculi) show a potassium-induced respiratory increase of 30-40 per cent (Hertz & Clausen, 1963). Slices from cerebrum, cerebellum and basal ganglia (e. g. Hertz & Clausen, 1963; Ridge, 1967) show a distinct rise in oxygen consumption during exposure to potassium-rich media. A similar response has been reported in the excised cervical sympathetic ganglion by Nagata (1969) and in the retina by Tamarit & Gallego (1962), whereas Dickens & Greville (1935), Vrba & Folberger (1958) and Cohen & Nöell (1960) observed a respiratory decline in the latter preparation.

The structures which show an increased rate of oxygen uptake in potassium-rich media, e. g. cerebral cortex, cerebellar cortex, basal ganglia and brain stem, react in a similar way to electrical stimulation (McIlwain, 1952b). Application of electrical pulses seems, however, also to lead to a respiratory increase in preparations which are insensitive to high concentrations of potassium, since a considerable increase in the respiratory rate has been observed with peripheral nerve (Brink, Bronk, Carlson & Connelly, 1952) and spinal cord (Winterstein, 1908). Labilizers resemble electrical pulses in increasing the metabolic rate in peripheral nerve (Hill, 1933; Schmitt & Gasser, 1933; Schmitt, 1936).

b. Homogenates and sieved cells: The failure of high concentrations of potassium (and of electrical pulses or drugs) to increase the oxygen uptake by brain homogenates is a repeated finding in the literature (table 2 in Elliott & Libet, 1942; Nara-

yanaswami & McIlwain, 1954; Ghosh & Quastel, 1954; Wollenberger, 1955a; Jones & Banks, 1970a), but dinitrophenol (Wollenberger, 1955a) and possibly also calcium lack (table 2 in Elliott & Libet, 1942) may increase the rate of oxygen uptake in homogenates.

The cell suspension obtained by passing brain cortex cubes through nylon sieves reacts to addition of 60 mM potassium with a considerable increase in respiratory rate (Kováru - Mlejnková, 1971).

c. Isolated cells: The results obtained with slices or sieved cells allow no conclusions whether it is the glial cells or the neurons (or both) which react metabolically to an increased potassium concentration. Micromanometric measurements (Fig. II, 7) have shown that the oxygen uptake by microdissected nerve cell bodies continues unaffected when a high concentration of potassium is added, whereas corresponding glia cell clumps show an initial increase of the respiratory rate (Hertz, 1966; Aleksidze & Blomstrand, 1969). This stimulation is very short-lasting, and it is followed by an increased rate of respiratory decline. This finding is at variance with the report by Bradford and Rose (1967) that both a "neuronal-enriched" and a "glial-enriched" cell fraction show a potassium-induced stimulation of oxygen uptake of 20-30 % (cf. also Ruscak et al., 1967). Using the somewhat different isolation procedure described by Blomstrand and Hamberger (1969, 1970), Haljamäe and Hamberger (1971) have, however, confirmed the neuroglial localization of the potassium effect; addition of potassium (50 mM) to their "glial-enriched" fraction caused a respiratory stimulation of 85 %, whereas the corresponding response by the "neuronal-enriched" fraction was only 15 %. The oxygen consumption by cultivated neuroblastoma cells or neurons from the brain is also not increased by excess potassium (Nissen et al., 1973), whereas cultivated glia cells react metabolically to the addition of potassium (Hertz, Dittmann, Sensenbrenner & Mandel, 1973). The ontogenetically late appearance of the potassium-induced stimulation (II B, 2a) is in accordance with a neuroglial - and probably astrocytic - localization (I, B, 4).

d. Subcellular particles: In view of the absence of a potassium-induced stimulation of oxygen uptake in homogenates it seems peculiar that high concentrations of potassium may increase the respiratory rate of isolated brain mitochondria (Sugawara &

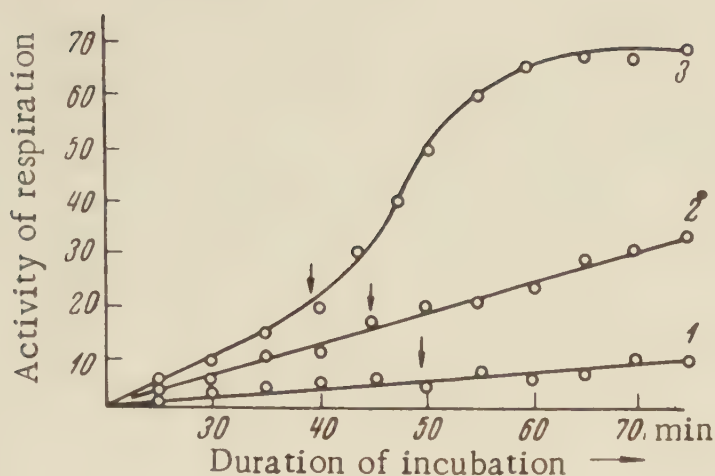


Fig. II, 7. Cumulative rates of oxygen uptake by neurons (2) and neuroglia (3) from Deiters' nucleus in the rabbit compared to the apparent respiration in control divers (1). Excess potassium was added at arrows without opening the divers (L. Hamberger, 1968). The respiration is shown in arbitrary units (change in gas volume of flotation chamber (mm burette) to maintain buoyancy of diver).

From Aleksidze & Blomstrand, 1969.

Utida, 1961; Utida & Sugawara, 1963; Krall, Wagner & Gozansky, 1964; Ozawa, Seta, Araki & Handa, 1967). The possibility of a contamination of many brain mitochondrial preparations was pointed out by Opit & Charnock (1965), but Clark and coworkers (Clark, 1970; Clark & Nicklas, 1970; Nicklas, Clark & Williamson, 1971) confirmed that 100 mM potassium cause a distinct increase in the rate of oxygen uptake by a "brain mitochondrial preparation known to be relatively free from synaptosomal and cytoplasmic contamination". In contrast to the potassium-induced respiratory stimulation in brain slices no presence of sodium was required, the response was not abolished by uncoupling and the content of ATP was unaffected (cf. III B, 2a). The metabolic response was also not directly connected with a concomitant swelling (cf. IV B, 2b), and the effect could be observed with succinate as the only substrate (cf. VI B, 2c).

The rate of oxygen uptake by synaptosomes is increased by electrical stimulation (De Belleruche & Bradford, 1972).

3. pH effects on stimulated and unstimulated respiration

Most tissues show a maximum respiratory intensity around a medium pH of 7.5. This is also the case with brain homogenates (Elliott & Birmingham, 1949), but not with brain slices, which show their greatest rate of oxygen uptake around a pH of 9.0 (Canzanelli, Greenblatt, Rogers & Rapport, 1939; Elliott & Birmingham, 1949). In contrast to other procedures which increase the "resting" metabolism (e.g. exposure to certain drugs (II B, 2a) a moderate increase in pH seems also to increase the potassium-stimulated respiration (L. Hertz, unpublished results).

4. Anion effects

5-10 % CO₂ increases the respiration by retina (Craig & Beecher, 1943; cf. also Svaetichin, Negishi, Fatechand, Drujan & Selvin de Testa, 1965), and may have a similar effect on brain tissue (Ashford & Holmes, 1931; C. S. Kjeldsen & L. Hertz, unpublished experiments). Most investigators have, however, reported that the presence of CO₂ is of little or no importance for the oxygen consumption by homogenates, chopped brain or brain-cortex slices (incubated in "balanced" or potassium-rich media) at physiological pH (Elliott & Libet, 1942; Craig, 1944; Birmingham & Elliott, 1948; Elliott, 1955; Weiss et al., 1972). That CO₂/HCO₃⁻ seems to play an important physiological role is seen by the fact, that the potassium-induced swelling may be prevented by the carboanhydrase inhibitor acetazolamide (Bourke & Nelson, 1972; cf. also Kimelberg & Bourke, 1973).

Replacement of all chloride in the medium with nitrate or isethionate has no major effect on the oxygen consumption by brain slices (Thomas & McIlwain, 1956; Bourke & Tower, 1966a), or brain homogenates (Elliott & Libet, 1942), whereas substitution with sulphate or phosphate may lead to an increased rate of oxygen consumption (Elliott & Libet, 1942; cf. however also Gore & McIlwain, 1952). The possibility of precipitation of calcium should, however, be kept in mind. In muscle, the rate of oxygen uptake is increased if chloride is replaced by either NO₃⁻, Br⁻, SO₄⁻, I⁻ or SCN⁻ (Chang et al., 1935; Novotný & Vyskocil, 1965; cf. also Hill & Howarth, 1957).

Certain polyvalent anions (i.e. gangliosides) are able to restore the reactivity to electrical pulses in brain slices after it has been abolished by protamine, a basic protein (McIlwain, 1961, 1963, 1964). This is probably due to their binding with the protein. Protamines are also given off from the cells during incubation of slices in cold media (McIlwain, 1959; Wolfe & McIlwain, 1960, 1961), which abolishes the response to electrical stimulation (Marks & McIlwain, 1959).

C. DISCUSSION

The oxygen uptake by brain slices incubated in a potassium-rich, glucose-containing, oxygenated medium is relatively close to that in the functioning brain in vivo (II A, 3 - cf. table II, 2). This may be related to the fact that the brain continuously is exposed to afferent stimulation (cf. Elliott, 1957) and may indicate that the potassium-induced stimulation (and that evoked by electrical pulses) reflects a mechanism which is of major importance for the function of the central nervous system. It further shows that brain slices constitute a suitable preparation for study of this mechanism. Homogenates, in contrast, do not show an increased rate of oxygen consumption in potassium-rich media (or during exposure to electrical pulses) and have accordingly lost a characteristic which may be fundamental in connection with the function of the brain. Peculiarly enough, isolated mitochondria respond to the increased potassium concentration with an increased oxygen consumption, but the differences between the reaction by brain slices and by mitochondria (with respect to substrate specificity, sodium requirement, drug effects and relation to effect on ATP content and swelling) indicate that different mechanisms must be at work.

The response by glia cells to excess potassium resembles that in slices since the stimulation of oxygen consumption is accompanied by a decrease in ATP content (III B, 2a) and an increased swelling (IV B, 2b). The neuroglial localization of the response has been verified by several different techniques, but is only compatible with the absence of a potassium effect on respiration in peripheral nerve and central white matter on the assumption that metabolic differences are found between different types of glial cells, e.g. those occurring in typical grey and white matter. This assumption (cf. also Waelsch 1960; Tower & Bourke 1966; Hertz 1966, 1969) may invalidate general conclusions about neuroglial metabolism based upon findings in white matter or preparations containing reactive glia cells (cf. II B, 1b). Evidence was found that also a considerable part of the resting oxygen uptake should occur in glia cells, but it has been pointed out (e.g. Bunge, 1970; Marchbanks, 1970; Johnston & Roots, 1972) that the respiratory activity in neuropil samples mainly could be due to the presence of neuronal processes showing a high respiratory intensity (cf. Lowry et al., 1954). The relatively high rate of oxygen uptake by cultivated astrocytes (with little neuronal contamination - cf. I B, 3) seems, however, unequivocally to indicate that a considerable fraction of the "unstimulated" metabolism must occur in glial cells. The good concordance between respiratory rates per volume in microdissected neuropil clumps and in cultivated astrocytes furthermore supports the concept that the contamination of neuropil samples with neuronal expansions is of little importance for the metabolic activity.

The "resting" and the "stimulated" respiration seem to differ from each other in several respects. Choline may replace sodium in the maintenance of the respiration but not in its stimulation, and the stimulated respiration is specially sensitive to high concentrations of magnesium (II B, 2a) and to certain drugs (II B, 2a). A plausible explanation of the potassium-induced stimulation of oxygen uptake may be that it is evoked by stimulation of an enzyme system like the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ with excess potassium (II B, 2a; VI B, 4c). Also the stimulation evoked by electrical pulses, veratrine or glutamate may conceivably be due to local increases in the potassium concentration, since both electrical stimulation and addition of veratrine or glutamate lead to a release of potassium ions from the cells (IV B, 3b), and the extracellular concentration of potassium accordingly may increase (I B, 2; IV C). The external surface of the cell membrane, which probably possesses the potassium-sensitive site (cf. Ruscák & Whittam, 1967; Marchbanks, 1970) may thus become exposed to a high potassium concentration during both the electrical, the drug-induced and the potassium-induced stimulation of oxygen consumption. The almost immediate onset of the latter phenomenon (II B, 2a) is in accordance with an extracellular effect, but it has also been suggested that the metabolic stimulation evoked with electrical pulses or glutamate should be due to the increase in the internal sodium concentration (Keesey, Wallgren & McIlwain, 1965; Swanson & McIlwain, 1965; cf. also Bradford & McIlwain, 1966 and IV B, 3a). In either case it is difficult to explain the respiratory stimulation by glutamate in the absence of sodium (II B, 2a), but certain amino acids have been found capable of maintaining a certain content of potassium in sodium-free media (IV B, 3b). The concept of a close correlation between the potassium-induced and the electrical stimulation is supported by the several analogies, which exist between the two phenomena, e.g. the dependence of both upon the presence of sodium and their abolishment by homogenization (II B, 2b). Differences are also found, e.g. with respect to drug sensitivity (II B, 2a), phosphate metabolism (III B, 4a), intermediary metabolism (VI B, 3b), and stimulation of peripheral nerve. If the electrical stimulation is secondary to changes in ion concentrations, atropine, chlorpromazine and tetrodotoxin may work by preventing these alterations (IV B, 3a-b) and thus counteract the electrical, but not the potassium-induced increase of oxygen uptake. Protamine seems to affect mechanisms close to the point of action by high concentrations of potassium and by electrical pulses as indicated by the inhibition of either of these two stimulatory agents, but not of the high rate of oxygen uptake evoked by dinitrophenol.

III

Phosphate Metabolism

A. PHOSPHATE METABOLISM IN BRAIN

1. Phosphorus fractions

a. Acid-soluble phosphorus: Chemical energy derived from metabolic break-down is stored and utilized as high-energy compounds, primarily ATP and phosphocreatine. These are contained in the acid-soluble phosphorus fraction, i.e. the phosphate fraction which is readily extracted by dilute acid and accounts for about 1/4 of the total phosphorus in brain (Heald, 1960, p. 79; McIlwain, 1966, p. 36).

b. Acid-insoluble phosphorus: The acid-insoluble phosphorus fraction accounts for about 3/4 of the total brain phosphorus and comprises phospholipids and phosphoproteins together with nucleic acids and "residual organic phosphorus" (Heald, 1960, p. 88-96). With brain slices it has been found that the incorporation of inorganic ^{32}P into at least the majority of these compounds is dependent upon oxidative phosphorylation (e.g. Findlay, Rossiter & Strickland, 1953; DeLuca, Rossiter & Strickland, 1953; Findlay, Magee & Rossiter, 1954; Strickland, 1954). The metabolism of the acid-insoluble phosphates is accordingly closely dependent upon that of the acid-soluble phosphates.

2. Phosphate metabolism in vivo

The acid-soluble phosphorus compounds which have been most thoroughly studied in the brain are phosphocreatine and the phosphonucleotides, primarily ATP. The in vivo level of both phosphocreatine and ATP is around 3 μ moles/g fresh tissue (McIlwain, 1966 p. 36; Fölbergrová, Passonneau, Lowry & Schultz, 1969; Granholm, Lukjanova & Siesjö, 1969). Hypoxia, insulin hypoglycemia, and electrically or chemically induced convulsions cause a decrease of these compounds (McIlwain, 1966, p. 40), whereas barbiturates lead to an increase and furthermore prevent the decrease normally observed in hypoglycemia.

The loss of electrical brain activity and of excitability which may be induced by hypoxia occurs before the level of the "energy-rich" phosphates has fallen drastically (Gurdjian, Webster & Stone, 1947; Albaum, Noell & Chinn, 1953 - cf. also Richter, 1955).

3. Phosphate metabolism in vitro

The concentrations of both phospho-creatine and ATP become greatly reduced almost immediately after the death and are accordingly low in freshly cut slices. However, some 1/2 to 3/4 of the in vivo level may rapidly (i. e. within 10 min.) be restored during incubation with oxygen and substrate (e.g. McIlwain, Buchel & Cheshire, 1951; McIlwain, 1952a, 1966, p. 66; McIlwain & Gore, 1953; Swanson, 1968; Jones & Banks, 1970b; Okamoto & Quastel, 1970a). A comparable content of ATP may be found in cultivated neurons and astrocytes (Schousboe et al., 1970). Isolated nerve cells without surrounding glia cells are able to incorporate inorganic phosphate into energy-rich phosphates (Hillman & Hyden, 1965b).

The turnover of inorganic phosphate into certain acid-insoluble phosphates (e.g. phosphatidyl-inositol) is fast (McIlwain, 1966, p. 205), but drastically decreased after homogenization (Findlay et al., 1953; Findlay, Strickland & Rossiter, 1954; Strickland, 1954). Lowering of the oxygen tension leads to a reduced content of ATP (Yamamoto & Kurokawa, 1970). Exposure to anoxia leads to a marked decrease of the phosphocreatine level, and also the incorporation of ^{32}P into phospholipids, nucleic acids, and phosphoproteins becomes reduced (see, e.g. Heald, 1960, p. 107). This lowering of the phosphocreatine level may be irreversible in spite of an almost completely reversible effect on the rate of oxygen uptake (Thomas, 1956; Heald, 1960, p. 104).

B. ION EFFECTS ON PHOSPHATE METABOLISM

1. Sodium

a. Acid-soluble phosphorus: A reduction of the concentration of energy-rich phosphates in sodium-deficient media has been described by Gore & McIlwain (1952), Rose (1965a) and Okamoto & Quastel (1970a), whereas Abadom & Scholefield (1962b), Margolis & Lajtha (1968) and Kato (1970) found the absence of sodium to be without effect. The incorporation of ^{32}P into ATP seems to be reduced when sodium is replaced with choline (Rose, 1965a).

b. Acid-insoluble phosphorus: The replacement of all sodium in the medium with choline diminishes the rate of incorporation of ^{32}P into, e.g. phospholipids with at least one third (Tsukada, Takagaki & Hirano, 1958; Brossard & Quastel, 1963; Rose, 1965a). In analogy with the importance of sodium as a co-factor by the "stimulated" respiration (II B, 2a), also the increased incorporation of ^{32}P into acid-insoluble phosphates which is evoked by high concentrations of potassium (III B, 2b) or by acetylcholine (see III B, 6) requires the presence of a minimum amount of sodium (Tsukada *et al.*, 1958a; Yoshida & Nukada, 1961; Brossard & Quastel, 1963). The stimulation of the incorporation which may be evoked by lack of calcium (III B, 3) has, in contrast, been observed even in the absence of sodium (Brossard & Quastel, 1963).

2. Potassium

a. Acid-soluble phosphorus: The exposure to about 100 mM potassium leads almost immediately to a considerable decrease in the levels of phosphocreatine and ATP in brain slices (McIlwain, 1952a; Gore & McIlwain, 1952; Brossard & Quastel, 1963; Rolleston & Newsholme, 1967; Biesold, 1967), and the threshold concentration of potassium is about 25 mM (Huttunen, 1969; Takagaki, 1972). A minimum concentration of sodium is required for the response, and in potassium-rich media the level of energy-rich phosphates may be higher in the absence of sodium than in the presence of this ion (Gonda & Quastel, 1966). A concomitant increase in the concentration of inorganic phosphate is retarded by about 20 seconds and provides

evidence that the inorganic phosphate is not directly derived from the acid-soluble phosphates (Heald, 1954). Simultaneously the rate of incorporation of ^{32}P into acid-soluble phosphate is increased by potassium (Brossard & Quastel, 1963; cf. however also Fonyó, Kovach, Maklary, Leszkovsky & Meszaros, 1958). The potassium-induced decrease in ATP content seems to be specific for glia cells (astrocytes), since addition of 50 mM potassium to cultivated neurons from the dorsal root ganglion of the chick embryo has no effect on the content of ATP (Schousboe et al., 1970).

Also omission of potassium from the medium leads to a reduced content of ATP (Margolis & Lajtha, 1968; Okamoto & Quastel, 1970a).

b. Acid-insoluble phosphorus: Some discrepancies are found in the literature regarding the effects of increased potassium concentrations on incorporation of ^{32}P (measured as counts per min per g (mg, ng) tissue or phosphate) into phospholipids, phosphoproteins, and nucleic acids. Both stimulatory and inhibitory effects have been described, and it was suggested by Heald (1960, p. 132) that the length of the incubation period is of importance, i. e. that a relatively short period of incubation in potassium-rich media leads to a stimulation (e.g. Tsukada et al., 1958a; Tsukada, Nagata & Hirano, 1960), whereas prolonged exposure causes a decrease of the phosphate incorporation (e.g. Findlay et al., 1954a; cf. also Rose, 1965a). More recent investigations (Brossard & Quastel, 1963) in which the incorporation was followed during the incubation, have verified such an initial augmentation and subsequent reduction of the tissue radioactivity compared to that obtained during incubation in a "balanced" medium (see Fig. III, 1), and it was suggested that this effect by high concentrations of potassium may be analogous to that exerted on oxygen consumption (cf. II B, 2a). - It must, however, be kept in mind that the uptake of a radio-isotope occurs as a first order reaction (cf. IV A, 3e), provided the tissue concentration of the compound is kept constant. The radioactivity in the tissue shows accordingly no further increase once equilibrium has been obtained. It is therefore only the velocity with which this equilibrium is obtained which gives an estimate of the exchange rate. This may be indicated by the differences between the asymptotically approached radioactivity at equilibrium and the actual radioactivities measured at different times during the incubation. These differences are shown on a semilogarithmic scale in Fig. III, 2, and it can be seen that the phosphate exchange follows a strictly exponential course both in the "balanced" and in the potassium-rich medium. The rate of exchange in either medium is thus

constant during the period shown (0-120 min.), but it is considerably increased in the potassium-rich medium.

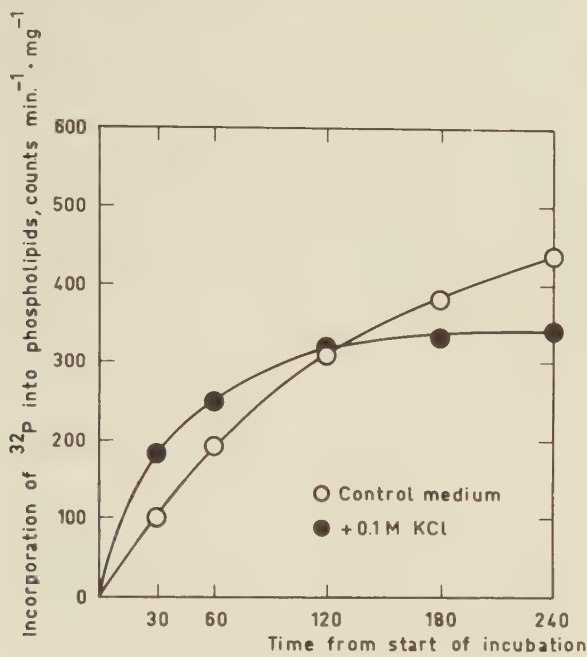


Fig. III, 1. Effects of 100 mM KCl on incorporation of ^{32}P into phospholipids of rat brain-cortex slices. The incorporation of ^{32}P is indicated as total counts per minute per mg wet wt. Abscissa shows time in minutes.
After Brossard & Quastel, 1963.

An easier and only slightly less accurate way of measuring exchange rates is to indicate the radioactivity after a short time of exposure to the radio-isotope, e.g. 30 min. (cf. Fig. III, 1). This was done by Brossard & Quastel (1963) who found a maximum stimulation (about 60 per cent above the resting level) to occur with 100 mM potassium. The response seems to be specific for the brain (Tsukada *et al.*, 1958a).

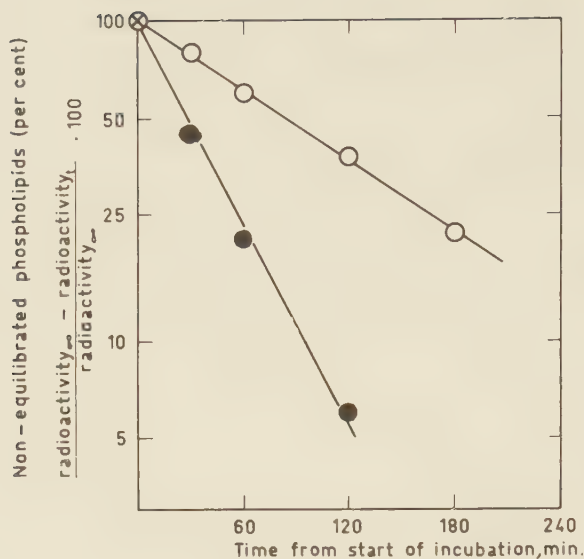


Fig. III, 2. The asymptotically approached radioactivities were estimated from Fig. III, 1 to 500, respectively 333 counts $\cdot$ min $^{-1}$ $\cdot$ mg $^{-1}$. The actual radioactivities were subtracted from the radioactivities at equilibrium, expressed as percentages of the latter, and plotted on a logarithmic scale as a function of time. Expressed in this way the uptake of radioactive phosphate is seen to occur as a first order reaction, and the half-times of the exchange may be read to 82 respectively 28 min in the "balanced" (o) and in the potassium-rich (●) medium.

3. Other cations (ammonium and calcium)

The ammonium ion resembles the potassium ion in increasing respiration (cf. II B, 2a) and glycolysis (cf. VI B, 3b), and it also diminishes the level of phosphocreatine (McIlwain & Gore, 1952). An increase in the ammonium concentration has, however, only been observed to cause a reduction and no augmentation of ^{32}P incorporation into phospholipids (Findlay *et al.*, 1954a) and into "protein-bound" phosphate (Tsukada *et al.*, 1958a).

Omission of calcium may cause a lowering of the phosphocreatine level (Gore & McIlwain, 1952) and a decrease of the incorporation of ^{32}P into e.g. phosphoproteins and phospholipids (Tsukada *et al.*, 1958a; Findlay *et al.*, 1954a; cf. however also Rose, 1965a). Minor differences seem, however, to exist between the effects exerted by excess potassium and by those evoked by calcium lack (Biesold,

1967; cf. also III B, 1b). Other authors (Brossard & Quastel, 1963) have described a stimulation of the incorporation of ^{32}P into phospholipids.

4. Electrical stimulation

a. Acid-soluble phosphorus: Application of electrical pulses causes a lowering of the level of acid-soluble phosphorus, but the time course of the decrease differs slightly from that observed after exposure to high concentrations of potassium, and the decrease in the ATP level may be only transient (Heald, 1954 - cf. however also Jones & Banks, 1970a). In the absence of sodium no decrease is evoked (Kato, 1970).

The rate of breakdown of phosphocreatine becomes as high as 1200-1400 $\mu\text{moles/g/hr.}$ initially during electrical stimulation of guinea pig brain slices (Heald, 1960, p. 115; McIlwain, 1966). As pointed out by Heald, this extremely high rate suggests that the breakdown is not only the result of an inhibition of oxydative phosphorylation. If the ATP/ADP ratio is not very drastically altered one would expect a rate of breakdown corresponding to the rate of oxygen uptake before the application of the pulses. This was 55 $\mu\text{moles O}_2/\text{g wet wt./hr.}$ and if the P/O ratio was 3.0 the total requirement of the tissue for "energy-rich" phosphates to maintain a steady state in the absence of oxydative phosphorylation could be met with by breakdown of 330 $\mu\text{moles/g wet wt./hr.}$

After the cessation of the pulses the previous level of phosphocreatine is rapidly (i.e. within 90 seconds) re-obtained (Heald, 1954). This is probably one of the processes mirrored in the stimulation of oxygen consumption which also continues beyond the actual period of stimulation (cf. II B, 2a).

b. Acid-insoluble phosphorus

b. Acid-insoluble phosphorus: Passage of electrical pulses for 10 seconds causes a considerable increase in the incorporation of ^{32}P into phosphoproteins (Heald, 1957), whereas the changes in phospholipids, nucleic acids and "residual" organic phosphorus are insignificant. The lack of effect on the latter compounds may represent a difference from the potassium-induced stimulation.

5. Drugs

Addition of dinitrophenol decreases the level of energy-rich phosphates (Abadom & Scholefield, 1962a; Okamoto & Quastel, 1970a) and diminishes the incorporation of ^{32}P into acid-soluble phosphate, phosphoproteins (Tsukada et al., 1958a) and phospholipids (Yoshida, Nukada & Fujisawa, 1961; Yoshida & Nukada, 1961). Veratrine and related drugs cause, in contrast, an increased rate of incorporation of ^{32}P into phosphocreatine together with a reduced concentration (Wollenberger & Wahler, 1956; Yoshida et al., 1963a). Some of the drugs which counteract the stimulation of oxygen uptake by electrical pulses or excess potassium also prevent the concomitant fall of phosphocreatine. This has been demonstrated with e.g. ethanol (Wallgren, 1963b), ouabain (Minakami, Kakinuma & Yoshikawa, 1963) and protamine (McIlwain et al., 1961). In the absence of stimulation ouabain (0.2 mM) may, however, evoke a decrease in the content of ATP (Banay-Schwartz, Piro & Lajtha, 1971). Anticonvulsants or cocaine in concentrations which inhibit the electrical stimulation of the oxygen uptake, have both been reported to have no effect on the decrease in the concentration of phosphocreatine evoked by electrical stimulation or glutamate (Greengard & McIlwain, 1955a; Woodman & McIlwain, 1961), and to exert some protection against the electrically induced reduction (Bollard & McIlwain, 1959).

6. Amino acids and transmitters

Glutamate resembles excess potassium in affecting the incorporation of ^{32}P into phospholipids (Findlay et al., 1954a; Tsukada, Nagata, Hirano & Matsutani, 1963; cf. however also Yoshida & Quastel, 1962), phosphoproteins (cf. Tsukada et al., 1958a) and other acid-insoluble phosphate fractions (Rose, 1965a), and in lowering the concentrations of phosphocreatine (McIlwain, 1952a; Woodman & McIlwain, 1961) and ATP (Abadom & Scholefield, 1962a; Rose, 1965a). The decrease in the phosphocreatine level is only observed with slices and not with homogenates, and occurs initially at a maximum rate of 50-85 $\mu\text{moles/g wet wt/hr}$ (Woodman & McIlwain, 1961; Bradford & McIlwain, 1966), i.e. considerably slower than the corresponding effect induced by electrical pulses (III B, 4a). The rate of phosphocreatine breakdown is relatively close to that of glutamine synthesis (cf. V A, 2b) which might indicate that the lowering of energy-rich phosphates could be due to the increased synthesis of glutamine (Acs, Balázs & Straub, 1953; Heald, 1960, p. 103; Woodman & McIlwain, 1961 - cf. however also Bradford & McIlwain, 1966). It seems, however, likely that also the active uptake of glutamate (and potassium - cf. IV B, 3b) from the glutamate-containing medium must contribute

to the increased metabolic rate (cf. Bradford & McIlwain, 1966). GABA causes an increased incorporation of ^{32}P into phospholipids (Tsukada *et al.*, 1963), and several amino acids lead to a decrease in the level of phosphocreatine. Aspartate exerts almost as marked an effect as D- and L-glutamate (cf. IV B, 3b and IV B, 4c), whereas glutamine causes only a minor decline (Woodman & McIlwain, 1961).

Acetylcholine resembles other stimulatory agents in causing an increase in the incorporation of ^{32}P into acid-insoluble phosphorus fractions, primarily phosphoinositide and phosphatidic acid (Hokin & Hokin, 1955; Hokin, Hokin & Shelp, 1961; Yoshida & Quastel, 1962). The stimulation may be observed both in peripheral ganglia and in slices from several structures of the brain, e.g. cerebral cortex and basal ganglia, but not in cerebellum which probably does also not contain cholinceptive synapses (Hokin *et al.*, 1961). The presence of sodium is indispensable (Brossard & Quastel, 1963).

In contrast to the stimulation of phosphate incorporation with high concentrations of potassium, omission of calcium or application of electrical pulses, a long period of incubation (i.e., several hours) is required to obtain the effect. Furthermore, unphysiologically high concentrations of acetylcholine (10^{-2} - 10^{-3} M) have to be used, and the response is inhibited by atropine (Brossard & Quastel, 1963). Another difference between acetylcholine and the other agents which increase phosphate turnover is that acetylcholine apparently does not alter the level of ATP in brain cortex slices (Brossard & Quastel, 1963), possibly reflecting the paucity of effect on oxygen consumption (cf. II B, 2a).

C. DISCUSSION

It is a fundamental question whether the decrease in the level of "energy-rich" phosphates evoked by e.g. high concentrations of potassium is a result of an increased utilization or a decreased production. The latter possibility seems of relevance since the concomitant augmentation of the oxygen uptake (II B, 2a) and lactate production (VI B, 3b) conceivably might be caused by an uncoupling of the oxidative phosphorylation (e.g. Lipmann, 1970). The quantitative considerations by Heald, give, however, evidence of an increased demand for energy during the electrical stimulation. Also the facts that high concentrations of potassium, in contrast to dinitrophenol (III B, 5), increase the rate of incorporation of ^{32}P into "energy-rich" phosphates, and that energy-requiring processes (e.g. incorporation of ^{32}P into organic phosphorus compounds) are stimulated, are against an uncoupling. The degree of correlation between ion effects on soluble and on insoluble phosphates is, however, unknown. The dependence on sodium and histological integrity by the potassium-induced stimulation of phosphate incorporation

into the acid-insoluble phosphorus fraction may indicate some connection between the latter phenomenon and the mechanisms lying behind the stimulated metabolism, whereas the relatively pronounced effects by acetylcholine on acid-insoluble phosphorus together with the almost negligible effect on energy metabolism seems to suggest a dissociation between ion effects on acid-soluble and acid-insoluble phosphates.

The difference between the potassium-induced stimulation and that evoked by electrical pulses with respect to the time course of the breakdown of "energy-rich" phosphates has been emphasized by Heald (1960) and by McIlwain (e.g. Discussion in Quastel, 1960). This difference might, however, be related to the fact that the potassium ions must diffuse from outside the tissue to their site of action during the potassium-induced stimulation, whereas the electrical pulses may act promptly and directly at the cellular level.

20-25 mM potassium seems to be the threshold value for potassium effects on both respiration (II B, 2a) and ATP content (III B, 2a), and the requirement for sodium in the potassium-induced or electrically induced breakdown of acid-soluble phosphate reminds strikingly of the similar dependence on sodium by the stimulated respiration. The confinement of the potassium-induced decrease in ATP content to glia cells is in accordance with the respiratory response (II B, 2c).

Ion Distribution and Transport

A. ION TRANSPORT AND CONTENT

1. Correlation between ion contents and membrane polarization

A major purpose of the metabolism in the brain probably is actively to secure a distribution of cations across its numerous membranes which is compatible with the function of the central nervous system. Since the gradients of certain ions (e.g. potassium) across cell membranes determine the electrical potentials of the cells, the ion transport provides means of influencing the neurophysiological activity. On the other hand membrane polarization is of importance in determining the ratio between intracellular and extracellular concentrations of ions which easily penetrate the membrane. If the intracellular concentrations of both chloride and potassium are regarded to be in electrochemical equilibrium with the external concentrations, one may write, at 37° (the Nernst equation)

$$E_m = 61 \log \frac{K_o^+}{K_i^+}$$

or

$$E_m = 61 \log \frac{Cl_i^-}{Cl_o^-}$$

where E_m is the membrane potential (in mV), 61 is the Nernst coefficient, and K_o^+ (Cl_o^-) and K_i^+ (Cl_i^-) are the concentrations of K^+ (Cl^-) outside and inside the cell. In cells which behave as perfect electrodes for both K^+ and Cl^- ,

$$\log \frac{K_o^+}{K_i^+} \text{ accordingly equals } \log \frac{Cl_i^-}{Cl_o^-}.$$

2. In vivo

a. Ion content: The ion concentrations in brain tissue removed immediately after the death of the animal are probably representative of those occurring in vivo (Hillman, 1964). The potassium concentration in adult grey matter is about $100\ \mu\text{moles/g}$ fresh weight (Yannet, 1940; Ames & Nesbett, 1958; Bachelard et al., 1962) and the sodium concentration is about half of this value (cf. also Grossmann, Lynch & Shires, 1968). In white matter the potassium concentration may be slightly lower, and the sodium concentration slightly higher, but the differences between grey and white matter are probably small (Stewart-Wallace, 1939; Levy, Herzog, Suzuki, Katzman & Scheinberg, 1965; Tower, 1969). In cortex from new-born animals the potassium and sodium concentrations are of approximately equal magnitude, i.e. $70\text{--}80\ \mu\text{moles/g}$ fresh weight (Franck, 1970). The Schwann cells of the squid nerve fiber have a high sodium concentration (Villegas, Villegas & Villegas, 1965; Villegas, 1968) and it has repeatedly been suggested that the sodium concentration should also be high in glia cells from the central nervous system (Gerschenfeld, Wald, Zadunaisky & De Robertis, 1959; Katzman, 1961; De Robertis & Gerschenfeld, 1961; Koch, Ranck & Newman, 1962; Hartmann, 1966, cf. also Franck, 1970). No conclusive evidence has, however, been reported, and findings in the leech (e.g. Nicholls & Kuffler, 1965; Kuffler & Nicholls, 1966) and in cultivated glia cells (IV A, 3c) do not support this concept.

Great differences are found between the concentration of e.g. potassium in marine and terrestrial species (Hillman, 1964), but the cation concentration shows no major interspecies variations in mammals (Pappius & Elliott, 1954). The chloride concentration has, in contrast, been found to increase with an increasing brain volume (Bourke, Greenberg & Tower, 1965), i.e. show an interspecies variation which is the inverse of that exhibited by nerve cell density (I B, 4) and acetylcholine content (V A, 2a), which conceivably might indicate a relatively high chloride content in glia cells. In the rat cerebral cortex the chloride concentration is about 30 and in the rabbit about $40\ \mu\text{moles/g}$ fresh weight (e.g. Bourke et al., 1965). It may be slightly lower in white matter (Tower, 1969).

In the cerebro-spinal fluid most ionic concentrations are relatively close to those in plasma but the potassium concentration is significantly lower, i.e. approximately 3 mM (Merrit & Fremont-Smith, 1938; Cooper, Lechner & Bellet, 1955; Bradbury & Davson, 1965; Reed, Withrow & Woodbury, 1967; Bourke, Nelson, Naumann & Young, 1970) and the chloride concentration higher, i.e. about 130 mM (Davson, 1967; Bourke et al., 1965, 1970).

b. Ion transport: The maintenance of constant ionic concentrations in the brain and the cerebro-spinal fluid is based upon a restriction to free movements between blood on one hand, and cerebro-spinal fluid and brain tissue on the other, known as the blood-liquor and the blood-brain barriers. These barriers are so effective that e.g. intraperitoneally or intravenously injected ^{42}K has scarcely reached equilibrium with the potassium in cerebro-spinal fluid and in brain after 18-24 hours (Noonan, Fenn & Haeger, 1941; Bradbury & Davson, 1965). Evidence is also found of an active removal of potassium from the cerebrospinal fluid (Domer & Whitcomb, 1964; Katzman, Graziani, Kaplan & Escrivá, 1965), whereas sodium may rather be secreted into the cerebro-spinal fluid (cf. Marchbanks, 1970).

The ionic movements between the cerebro-spinal fluid and the brain tissue seem, in contrast, to occur relatively unlimited (Bradbury & Davson, 1965 - cf. also Marchbanks, 1970).

c. Changes in ion content and transport: Electrical or chemical excitation of intact animals leads to a release of potassium and an uptake of sodium in the brain (Cicardo & Torino, 1942; Cicardo, 1945; Colfer & Essex, 1947; cf. also Marichich & Izquierdo, 1972). The changes in content of electrolytes and water involved in the development of cerebral edema are of considerable practical and theoretical interest, but a discussion of this subject seems to be beyond the scope of the present paper (see, e.g. Bakay, 1965; Pappius, 1965, 1969; Bourke & Tower, 1966a, b; Tower & Bourke, 1966; Tower, 1967; Bourke, 1969b; Bourke et al., 1970).

d. Extracellular and intracellular spaces: The concentrations of potassium, sodium and chloride in the brain cortex reported in IV A, 2a refer to the total weight of the tissue. To deduce intracellular concentrations, the amount of extracellular fluid must therefore be known. As discussed in I B, 2 the magnitude of the extracellular space of the brain in vivo is not known with certainty. Furthermore, a larger extracellular space has been observed in species with larger brain size than in those with smaller brain size (Bourke et al., 1965), and the extracellular space seems to decrease during maturation (Flexner & Flexner, 1949; Vernadakis & Woodbury, 1965; Ferguson & Woodbury, 1969; Caley & Maxwell, 1970, - cf. however also Pappius, 1969) In smaller adult mammals the extracellular space does probably not exceed 25% (e.g. Davson, 1967; Woodward, Reed & Woodbury,

1967; Oldendorf & Davson, 1967; Pappius, 1969; Marchbanks, 1970). The solid content accounts for another 20% in cortex (e.g. Pappius & Elliott, 1956a; Franck, 1970), and 30% in white matter (Reulen, Hase, Fenske, Samie & Schürman, 1970). The intracellular water thus accounts for at least 55% of the wet weight in cortical tissue. Ionic concentrations in the intracellular water phase (X) may be calculated (on the basis of a cortical extracellular space of 25%) using the equation (table IV, 1)

$$X = (a - b \cdot 25/100) 100/55$$

in which a is the concentration of the ion in the tissue (expressed per g final wet weight) and b its extracellular concentration. From a chloride concentration of 40 μ moles/g fresh weight in the rabbit brain (IV A, 2a), and an extracellular chloride concentration of 130 mM (IV A, 2a), a chloride concentration of 13.6 μ moles/g cell water can be deduced. The ratio between extra- and intracellular chloride concentrations is thus at most 10, whereas the corresponding ratio between the intracellular (about 150 mM) and extracellular (3-5 mM) concentrations of potassium is not less than 30 (table IV, 1). These "intracellular" concentrations are average concentrations and the chloride concentration depends heavily upon the estimate of the extracellular space area. 25% seems however to represent an upper limit for the extracellular space in the whole brain and there is no reason to expect an especially large extracellular space in cortex. Furthermore, Reulen *et al.* (1970) deduced an intracellular chloride concentration of about 60 μ moles/g cell water in brain cortex using ion contents and extracellular spaces determined in the same study.

e. Membrane potentials: The high potassium concentration in the brain is consistent with the presence of resting potentials up to -60 to -90 mV in neurons (Phillips, 1956; Takahashi, 1965; Lux & Pollen, 1966) and -80 to -95 mV in glial cells (Grossman & Hampton, 1968; Dennis & Gerschenfeld, 1969; Grossmann, Whiteside & Hampton, 1969); the average potential (Krnjević & Schwartz, 1967) seems to be considerably higher in the latter cell type (-62 mV) than in the former (-36 mV), but it is probably at present not possible to obtain experimental determination of the potential in the complicated network of neuronal and glial processes constituting the neuropil (I B, 1). If the chloride ion is in electrochemical equilibrium, an indirect estimate of the average polarization of the whole tissue might, however, be obtained from the ratio between intracellular and extracellular concentrations of chloride (IV A, 1) which is between 1/2 and 1/10 (IV A, 2d). The resulting

average intracellular potential is -20 to -60 mV. The latter value is compatible with the average potentials recorded in glial cells and in neurons. The average potassium equilibrium potential can correspondingly be calculated to at least -90 mV, i.e., about the highest membrane potential recorded. Both potassium and chloride ions can accordingly not be in electrochemical equilibrium in brain cortex in vivo. In vitro the discrepancy between the chloride and the potassium concentration ratios is still more pronounced (IV A, 3cd, IV B, 4a and Table IV, 1).

The effects of iontophoretic application of potassium or glutamate on membrane polarization in vivo are discussed in relation to the effects exerted by these agents in vitro (IV B, 4a and c).

f. Spreading depression: A peculiar neurophysiological phenomenon during which a wave of suppression of the normal electrical activity spreads from a stimulated point of the exposed brain over almost its entire surface is called spreading depression (Leao, 1944). The propagation is accompanied by a release of radioactive potassium ions from brain tissue which has been preloaded with ^{42}K (Brinley, Kandel & Marshall, 1960; Krivánek & Bures, 1960; Brinley, 1963; Vyskocil, Kriz & Bures, 1972), but no concomitant decline occurs in potassium content after a prolonged period of spreading depression (J. Bures, personal communication). A key role in the process has been attributed to the potassium ion (Grafstein, 1956) and to glutamate (V B, 1c). The localization of spreading depression reminds of that of the potassium-induced stimulation of respiration, since the phenomenon may be observed in the cerebral cortex, cerebellar cortex, basal ganglia (Fifková, Bures, Kostoyants, Krivánek & Weiss, 1961; Fifková, 1964) and retina (Gouras, 1958; Martins-Ferreira & De Oliveira Castro, 1966; Van Harreveld & Fifková, 1970), whereas it has not been reported with the spinal cord. Furthermore, the spreading depression shows certain metabolic analogies with the potassium-induced stimulation of oxygen uptake (e.g. Bures, Buresova & Krivánek, 1960; Ochs, 1962; cf. also Hertz, 1965a; Van Harreveld, 1966), and the two phenomena appear simultaneously (i.e., at about 14 days in the rat) during ontogenesis (Bures, 1957; Bures, Fifková & Mares, 1964). In younger rats a similar potassium release is evoked by the procedures normally leading to spreading depression, but the neurophysiological phenomenon itself is absent (Krivánek & Bures, 1960). This might conceivably indicate that the potassium loss primarily occurs from relatively mature (cf. I B, 4) nerve cell bodies, but that the glia cells are involved in the propagation (Hertz, 1965a, cf. however also Ochs, 1962; Bures et al., 1964). During spreading depression chloride ions seem to enter the apical dendrites (probably reflecting a de-

polarization) together with water (leading to a swelling) and sodium ions (Van Harreveld, 1958, 1966; Van Harreveld & Schadé, 1959; Van Harreveld & Khattab, 1967; cf. also IV C). The cell bodies of the astrocytes have, in contrast, been observed to shrink, but the volumes occupied by the astrocytic processes could not be measured (Collewyn & Schadé, 1965). "Idle", i. e. glial cells (cf. IV B, 4a) seem to become depolarized during spreading depression (Karahashi & Goldring, 1966).

3. In vitro

a. Spaces: For interpretation of measurements of ion and water content in brain slices it is desirable to know the volume of the extracellular (and thus also the intracellular) fluid. It may differ from the extracellular space in vivo (cf. IV A, 3b). Several substances, including sucrose (Davson & Spaziani, 1959), thiocyanate (e.g. Pappius & Elliott, 1956a), inulin (e.g. Harvey & McIlwain, 1968) or chloride (e.g. Tsukada, Hirano, Nagata & Matsutani, 1960; cf. also Pappius & Elliott, 1956a), which generally do not occur inside the cells in high concentrations, have functioned as indicator substances to measure the size of the presumably "extracellular" space. The terms "sucrospacespace", "thiocyanatespace", "inulinspace" and "chloridespace" have been used to designate the fraction of the tissue, generally expressed per unit fresh wet weight (cf. IV A, 3b), in which the indicator substance is found in the same concentration as in the medium provided its concentration in the remaining part of the tissue (the non-sucrose, non-thiocyanate, non-inulin, or non-chloride-space) is zero. Great caution should, however, be taken in correlating the exact magnitude of the non-sucrose, (non-thiocyanate, non-inulin, non-chloride) space with that of the intracellular compartment, and the magnitude of the sucrose- (thiocyanate-, inulin-, chloride-) space, with that of the extracellular fluid.

Both the sucrose (Bourke & Tower, 1966a) and the chloride space (Bourke, 1969a - cf. Fig. IV, 4) is independent on the "marker" concentration in the medium, but the chloride space is significantly larger than the sucrose (and inulin) space (e.g. Varon & McIlwain, 1961; Bourke & Tower, 1966a; Schousboe & Hertz, 1971a). This is probably because the chloride ion is relatively freely diffusible across the cell membranes in brain-cortex slices (Thomas & McIlwain, 1956; Bourke & Tower, 1966a), and the intracellular chloride concentration accordingly will be high in depolarized cells (Gibson & McIlwain, 1965; cf. also IV A, 3c and IV B, 3c); the same consideration may possibly apply to the use of thiocyanate as an indicator of the interstitial space (Marchbanks, 1970, cf. however also IV A, 3b).

In slices from new-born rats the sucrose and inulin spaces are identical and constitute about 35% (compared to a chloride space of 45%) which may represent the extracellular space relatively well (Schousboe & Hertz, 1971a; cf. however also Franck, 1970). In the slices from adult rats the sucrose space is, in contrast, significantly greater than the inulin space (e.g. Pappius & Elliott, 1956a; Pappius, Klatzo & Elliott, 1962; Pappius, 1965; Schousboe & Hertz, 1971a - cf. however also Bourke & Tower, 1966a). This might either suggest an incomplete filling of the extracellular space by inulin or uptake of some sucrose into the intracellular compartment (cf. Hertz, Schousboe & Weiss, 1970). Since the inulin space is approximately the same in slices from the immature and the mature brain (Franck, 1970; Schousboe & Hertz, 1971a), and the extracellular space, if anything, probably decreases with maturation (IV A, 2d, cf. however also Franck, 1970, p. 120) there is no reason to suppose an incomplete filling in the slices from the adult animals (cf. however Bourke & Tower, 1966a). Kinetical analysis of the efflux of the tracer (Fig. IV, 1) has shown that the washout of both sucrose and - to a minor extent - inulin follows a curve indicating the presence of at least one, slowly exchanging and one greater, rapidly exchanging compartment. The half-time of the rapidly exchanging inulin fraction is about 5-10 min (Schousboe & Hertz, 1971a; Lund-Andersen & Hertz, 1973), which for a slice thickness of about 0.5 mm corresponds to an apparent diffusion coefficient of $4 - 8 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ (cf. Schoffeniels, 1967). The "free diffusion coefficient" of inulin is about $13 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ (Phelps, 1965) suggesting that this component probably represents the extracellular compartment (cf. also Bourke & Tower, 1966a), and that the hindrance for free diffusion of inulin is only small, which is in agreement with previous conclusions by McLennan (1957). Similar, relatively slight inhibition of free diffusion has been observed in other tissues (for references, see Niedgergerke, 1957; McLennan, 1957). The presence of the slowly exchanging fractions may thus indicate that both markers have access to at least one intracellular compartment (Schousboe & Hertz, 1971a, Lund-Andersen & Hertz, 1973; cf. however also Bourke & Tower, 1966a; Schoffeniels, 1967, p. 19). This seems also to be the case in the leech central nervous system (Nicholls & Wolfe, 1967), whereas sucrose only penetrates one, extracellular compartment in the rabbit superior cervical ganglion (Woodward, Bianchi & Erulkar, 1969). The concept of a cellular uptake of extracellular markers in brain slices is supported by studies on temperature effects on the magnitude of the marker space (Cohen, Blasberg, Levi & Lajtha, 1968; Cohen & Lajtha, 1969, 1970, 1971) and by the dependence of the sucrose space upon the time during the incubation when the slice is exposed to the marker (Bourke & Tower, 1966a). All markers of extracellular space in brain-cortex slices from adult mammals seem thus to yield too large estimates (cf. Schousboe & Hertz, 1969). If an "extracellular" marker

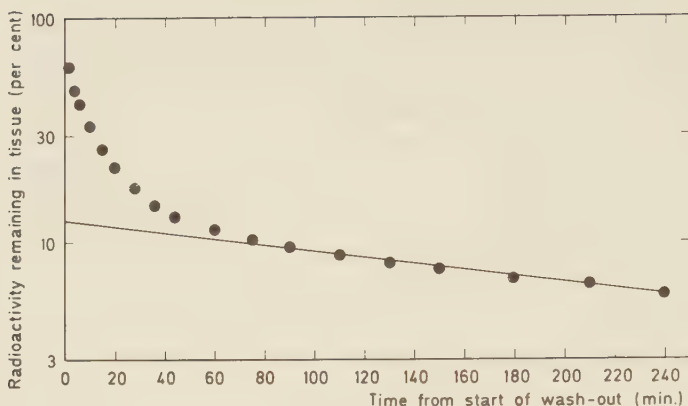


Fig. IV, 1. Wash-out of $[^3\text{H}]$ methoxyinulin from rat brain-cortex slices into a non-radioactive, "balanced", oxygenated medium. The slices were preincubated for 1 hr in an oxygenated medium containing the marker, and the amount of radioactivity remaining in the tissue at different times after the start of the wash-out is shown on a logarithmic scale.

The fully drawn line indicates the terminal, approximately straight part of the wash-out curve (fitted by eye) and its extrapolation to zero time. By subtraction of this line from the observed course, a line may be obtained, which shows the wash-out from a more rapidly exchanging fraction. Results are means of values obtained in three experiments. After Schousboe & Hertz, 1971a.

nevertheless is sought, the inulin space probably approximates the extracellular space most closely (Schousboe & Hertz, 1971a; cf. also Marchbanks, 1970), but as a further complication inulin does not have a uniform molecular weight (Phelps, 1965, cf. also Bourke & Tower, 1966a). The relatively great differences in the absolute values of the inulin spaces observed by different investigators using more or less different preparations or experimental conditions, and the effect of the temperature as well as of ionic deviations in the medium (IV B, 1), make it advisable to estimate the inulin (and non-inulin space) under the exact conditions which are used, e.g. to investigate the uptake of a substance into the supposedly intracellular fraction (Marchbanks, 1970).

b. Swelling: It is a prerequisite for discussion of ion distribution and transport (or distribution and transport of other compounds, e.g. amino acids) during in vitro

experiments that the content of the substance in the incubated tissue is expressed in relation to an unchanging weight unit. Great difficulties have accordingly arisen from the tendency of brain slices to take up water during the incubation. This so-called "swelling" which even under relatively good conditions during the incubation may amount to 40-50% (cf. below), introduces a great difference between the fresh and the final wet weight.

The swelling is generally expressed as the percentage increase of the fresh wet weight resulting from exposure to the medium and incubation. If the fresh wet weight for methodological reasons has not been determined (i. e. to initiate the incubation as fast as possible) it may be calculated on the basis of the final content of solids, which after incubation of rat brain-cortex slices in an ordinary, aerated medium has been found to constitute between 18 (Franck, Cornette & Schoffeniels, 1968; Franck, 1970; Lund-Andersen & Hertz, 1970) and 19% (Pappius & Elliott, 1956a) of the fresh wet weight. Variations within this range are of negligible importance for the calculation of both swelling and spaces (IV A, 3a). A value of 18.5% used by Schousboe & Hertz (1971a) was deduced on a somewhat different basis (defining the swelling as the percentage increase from the initial, incubated weight), but its position in the middle of the range given above, make the results directly comparable with the deductions based upon the other calculated or measured fresh weights. Already 10 seconds of contact with a "balanced" medium may lead to swelling of between 3 (Franck, 1970) and 5-10% (Varon & McIlwain, 1961; Pappius et al., 1962; Keesey et al., 1965; Bourke & Tower, 1966a), and during preparation of the tissue in poorly oxygenated media the swelling increases with about 2.5% per minute (Varon & McIlwain, 1961; Bourke & Tower, 1966a). In the course of the subsequent incubation at 37° under optimum conditions in an oxygenated, "balanced" medium the tissue may keep its weight constant for hours (Bourke & Tower, 1966a; Franck et al., 1968; Franck, 1970; Lund-Andersen & Hertz, 1970) leading to a total swelling of only 10-15%. A further swelling to 40-50% is, however, often encountered (e. g. Pappius & Elliott, 1956a; Varon & McIlwain, 1961) and anaerobiosis or glucose deficiency increases the swelling considerably (Pappius & Elliott, 1956a; Bourke & Tower, 1966a; Franck et al., 1968; Okamoto & Quastel, 1970a), whereas dinitrophenol only causes a slight increase of the swelling (Okamoto & Quastel, 1970b). The effect exerted by excess potassium is discussed in IV B, 2b.

Originally the swelling was supposed to occur extracellularly (cf. Turner, Eggleston & Krebs, 1950; Pappius & Elliott, 1956a, b) but more recent studies have shown that it is not exclusively or even not predominantly due to an increase in the extra-cellular water phase of the tissue. The fluid which is taken up almost immediately after exposure to a "balanced" medium seems to be accessible to inulin (and thiocyanate and sucrose) and may represent an increase in the ex-

tracellular volume (Lund-Andersen, unpublished experiments), adhering medium or a damaged part of the slice (Pappius & Elliott, 1956a; Varon & McIlwain, 1961; Pappius et al., 1962; Keesey et al., 1965; Pappius, 1965). The increased swelling, which occurs during incubation under sub-optimum conditions (including anoxia) is, in contrast, not combined with any augmentation of the inulin- or sucrose-space (Pappius & Elliott, 1956a; Varon & McIlwain, 1961; Pappius et al., 1962; Bourke & Tower, 1966a; Franck et al., 1968; Franck, 1970). The thiocyanate- and chloride spaces seem, however, to be increased (Pappius & Elliott, 1956a; Pappius et al., 1962; Bourke & Tower, 1966a), and it has been suggested, that the space permeable to these markers but not to inulin may represent glia cells (Pappius et al., 1962; Tower & Bourke, 1966). Provided the cells are easily permeable to chloride this concept does not seem to be in agreement with a high potential in glia cells (IV A, 3a and d), and the chloride and the thiocyanate spaces may possible also react differently to addition of excess potassium (IV B, 1). The concept is, however, supported by the finding that no swelling of neurons has been observed electronmicroscopically after incubation of brain slices in a "balanced" medium whereas astrocytes and their processes are greatly swollen (Gerschenfeld et al., 1959; De Robertis & Gerschenfeld, 1961, cf. however also Allen, 1955; Pappius et al., 1962). It is in further support of a neuroglial localization of the swelling that brain slices from new-born kittens or rats (which have few glia cells - cf. I B, 4) do not swell (Tower & Bourke, 1966; Okamoto & Quastel, 1970a; Schousboe & Hertz, 1971a). The parallelism between the ontogenetic development of the swelling and of the astrocytic development has been pointed out by Franck (1970).

c. Ion concentrations: The concentration of potassium found in vitro is lower than that occurring in vivo, whereas the sodium and chloride concentrations are higher.

Potassium: The reason for the low concentration of potassium during incubation in vitro is a pronounced loss of this ion during and immediately after the preparation of the tissue; the loss is followed by a more or less complete reaccumulation (e.g. Krebs, Eggleston & Turner, 1951; Pappius & Elliott, 1956b; Bachelard et al., 1962; Israel, Kalant & de Blanc, 1966; Bourke & Tower, 1966b; Franck, 1970).

The highest potassium concentrations (table VI, 1) in brain-cortex slices from adult rats after incubation in "balanced" media (containing about 5 mM potassium) seem to be those of 70-75 μ moles/g final wet wt. obtained by Franck et al., (1968), Franck (1970), and Lund-Andersen & Hertz (1970) using a specially designed

microtome (Franck *et al.*, 1968) and massive oxygenation (cf. also Arnfred, Hertz, Lolle & Lund-Andersen, 1970). The importance of a maximum supply of oxygen (or the concomitant mechanical agitation) is shown by the finding that a potassium concentration of only 60 μ moles/g final wet wt. was obtained after incubation in an ordinary Warburg-apparatus with CO₂/oxygen as the air phase (H. Lund-Andersen & L. Hertz, unpublished experiments; Franck, 1970). Under anoxic conditions the potassium concentration becomes still much lower (e. g. Pappius & Elliott, 1956b; Bourke & Tower, 1966b; Franck *et al.*, 1968; Lund-Andersen & Hertz, 1970). In brain-cortex slices from newborn rats the potassium concentration is significantly higher than in the mature tissue (Franck, 1970; Schousboe & Hertz, 1971b; Schousboe, 1972).

A relatively high content of potassium has been observed both in microdissected glia cell lumps (Hamberger & Röckert, 1964) in cultivated astrocytes (Lees & Shein, 1970) and in the "glia-enriched" fraction obtained by gradient centrifugation (Bradford & Rose, 1967; Haljamäe & Hamberger, 1971; Hemminki & Holmila, 1971), but not in isolated nerve cells (Hamberger & Röckert, 1964). The "neuronal-enriched" fraction shows little (Haljamäe & Hamberger, 1971; Rose & Sinha, 1969) or no accumulation of potassium (Bradford & Rose, 1967), but some uptake occurs in synaptosomes (Bradford, 1969; Escueta & Appel, 1969).

The potassium content in brain slices has often been expressed in relation to the fresh wet weight with a correction made for the swelling. To this end, the tissue has been considered as consisting of two compartments, i. e. one corresponding to the increase in weight during the incubation and the other to the fresh weight. The potassium concentration in the compartment formed during the incubation is regarded as identical to that in the medium (i. e. about 5 mM), and its content of potassium is calculated and withdrawn from the total measured amount of potassium in the tissue. The remaining, greater fraction of the potassium content is considered as restricted to the compartment corresponding to the fresh wet weight, and its concentration here calculated on this basis. The more recent demonstration that the swelling is not exclusively an extracellular phenomenon (IV A, 3b) implies that this correction can no longer be regarded as permissible. Corrections of this type are, however, so often performed that one must always ascertain the basis on which ionic concentrations in brain slices is expressed.

Calculation of "intracellular" potassium concentrations (or rather activities) is essential if correlations are sought between ion content and membrane polarization (IV A, 1). The uncertain determination of the extracellular space (cf. Table IV, 1), the possibility that the remaining, intracellular space may not be homogenous (cf. Schousboe & Hertz, 1971a) and the lack of knowledge of the activity coefficient inside the cells (Franck *et al.*, 1968) vitiates such a calculation. If any attempt nevertheless is made to estimate average concentrations, spaces

	in vivo	5 mM K ⁺	25 mM K ⁺	45 mM K ⁺	85 mM K ⁺
H ₂ O (ml/100 g final wet wt.)	80.0	84.6	85.8	87.4	87.2
Solids (g/100 g final wet wt.)	20.0	15.4	14.2	12.6	12.8
Inulin space (ml/100 g final wet wt.)	25.0	28.2	20.7	16.7	15.2
Total K ⁺ (μmoles/g final wet wt.)	100.0	67.8	82.8	83.2	111.9
Total Na ⁺ (μmoles/g final wet wt.)	50.0	81.7	80.4	97.4	98.4
Total Cl ⁻ (μmoles/g final wet wt.)	40.0	66.4	84.8	114.6	150.2
K ⁺ conc. (μmoles/g tissue water)	179.8	117.8	119.2	107.1	137.4
Na ⁺ conc. (μmoles/g tissue water)	45.4	77.6	80.4	105.9	108.2
Cl ⁻ conc. (μmoles/g tissue water)	13.7	53.7	83.4	122.4	164.8
E _M Cl ⁻ (mV)	-59.6	-23.0	-15.2	-8.4	-6.2
E _M K ⁺ (mV)	-95.1	-83.6	-41.4	-23.0	-12.4

Table IV, 1. Calculation of "intracellular" concentrations of potassium, sodium and chloride (μmoles/g tissue water) in rat brain-cortex slices incubated in media containing 5, 25, 45 or 85 mM potassium compared with the in vivo values. The in vitro results originate from experiments by Schousboe & Hertz (1971b and unpublished results) and the in vivo concentrations and extracellular space are those given in IV A, 2a, d for e.g. the rabbit. Using 100 mM Na⁺, 130 mM Cl⁻ and 5 mM K⁺ as the extracellular concentrations in vivo, average intracellular concentrations (x) were deduced from these values using the equation:

$$x = (a - b \cdot i/100)(100 - (i + c))^{-1} \cdot 100$$

in which a is the concentration of the ion in the tissue, b its extracellular concentration, i the inulin space and c the contents of solids. The equilibrium potentials (E_M) for chloride and potassium were calculated

Table IV, 1 (cont.)

from the intra- and extracellular concentrations by aid of the Nernst equation (IV A, 1). The uncertainty involved in such deductions of "intracellular" concentrations have been discussed, e.g. by Hertz, Schousboe & Weiss (1970) and Schousboe & Hertz (1971a). Since the extracellular space magnitudes probably are overestimated (cf. Lund-Andersen & Hertz, 1973) and a great extracellular space tends to give a low "intracellular" chloride concentration (and thus a high negative equilibrium potential) with only slight effect on the intracellular potassium concentration, the discrepancy between the potassium and the chloride equilibrium potentials is probably underestimated and seems thus to represent a real phenomenon.

and ion contents must be measured under totally identical experimental conditions (cf. IV A, 3a), and in table IV, 1, the inulin spaces reported by Schousboe & Hertz (1971a) are shown together with the concentrations of potassium (and other ions) and the contents of solids obtained by the same authors (Schousboe & Hertz, 1971b and unpublished experiments). On the basis of these values, average intracellular concentrations have been calculated as described in the legend of the table. It can be seen that the average intracellular potassium concentration after incubation in a medium with 5 mM potassium is about 120 μ moles/g tissue water corresponding to a ratio of 24 between the intracellular and the extracellular concentrations and thus to a resting potential of about -85 mV.

Sodium: A gain in sodium content occurs concomitantly with the potassium loss during and immediately after the preparation of brain slices. A subsequent extrusion of sodium seems more difficult to obtain than the corresponding uptake of potassium ions, but may be observed if the tissue is prepared with special care (e.g. Bachelard et al., 1962; Franck et al., 1968). Massive oxygenation seems of major importance to obtain as "physiological" a concentration as possible (Franck et al., 1968; Franck, 1970; H. Lund-Andersen & L. Hertz, unpublished experiments). Even then, the "average intracellular" concentration of 77 mM after incubation in a "balanced" medium (Table IV, 1) is considerably higher than the corresponding in vivo value.

Chloride: Also the concentration of chloride is normally higher in brain slices than in vivo (Cummins & McIlwain, 1961; Bachelard et al., 1962; Keesey et al., 1965), but it decreases rapidly to low values during incubation in chloride-deficient media (Thomas & McIlwain, 1956; Bourke & Tower, 1966a) indicating the absence of any pronounced tissue binding.

From table IV, 1 it can be seen that the average "intracellular" chloride concentration after incubation of rat brain-cortex slices in a "balanced" medium (containing 128 mM chloride) is 54 mM. Also Allen (1955), McIlwain and coworkers (Varon & McIlwain, 1961; Keeseey et al., 1965) and Bourke (1969a, b) have found that the total chloride "space" in brain slices greatly exceeds the extracellular space (cf. IV A, 3a). Though evidence has been presented, which may suggest an active uptake of chloride (IV A, 3e), the chloride ion is generally supposed to be passively distributed in accordance with the Nernst equation (see e.g. Pappius & Elliott, 1956a; Varon & McIlwain, 1961; Hillman, 1964; Keeseey et al., 1965; Gibson & McIlwain, 1965). In that case the chloride equilibrium potential of -23 mV should be representative of the potential in the cells constituting the non-inulin space.

d. Membrane potentials: Both potassium and chloride gradients between brain slices and the medium indicate the presence of membrane potentials (cf. IV A, 3c) but in the case of potassium the equilibrium potential was calculated to -85 mV, whereas the chloride distribution indicated a potential of -23 mV. This difference seems too large to be accounted for only by the fact that the neurons do not behave as perfect potassium electrodes and is thus compatible with the concept that mammalian glia cells differ from invertebrate glia cells with respect to membrane permeabilities (IV B, 4a). Direct measurements have shown the presence of membrane potentials of up to -80 mV in brain slices (Li & McIlwain, 1957; Hillman & McIlwain, 1961; Hillman, 1961; Gibson & McIlwain, 1965; Bradford & McIlwain, 1966), -40 mV in isolated neurons (Hillman & Hydén, 1965a) and -70 mV in glial cell cultures (Hild et al., 1958; Hild & Tasaki, 1962; Hild, 1964). The average values seem to be considerably lower (Li & McIlwain, 1957; Hillman & McIlwain, 1961), and attempts to record potentials from the "neuronal-enriched" fraction obtained by centrifugation have failed (Bradford & Rose, 1967). A special useful preparation for the study of glial membrane potentials is the packet glia cell of the leech (Fig. I, 7) which, due to its large size, is well suited for measurements of membrane potential (cf. however also I B, 4). Potentials of -60 to -90 mV have been obtained in this preparation (Kuffler & Potter, 1964) and in glial cells from the necturus optic nerve (Kuffler, Nicholls & Orkand, 1966; Kuffler & Nicholls, 1966).

In slices, the potentials appear during the first 30 minutes of incubation (Hillman & McIlwain, 1961), and they are much lower in the absence of a substrate or during hypoxia (Hillman, 1961; Hillman & Hydén, 1965a). Special precautions (e.g. careful preparation and slicing in situ - cf. Bachelard et al., 1962; Hill-

man, Campbell & McIlwain, 1963; Gibson & McIlwain, 1965) have often been taken to obtain a maximum polarization of the tissue, and it seems likely that only negligible membrane potentials exist in many brain slice preparations (IV B, 3c). In any case, it is only a minor part of the slice from which potentials may be recorded. In the experiments by Hillman & McIlwain (1961) and by Hillman et al. (1963) the electrode was thus in the regions of negative potentials during one fourth of its travel in the slice. Spike discharges were observed, but only in about 1 % of the penetrations (Hillman et al., 1963). Postsynaptic responses to electrical stimulation and provoked spike potentials have, however, been obtained in olfactory cortex slices (cf. IV B, 4b).

e. Ion transport: Both the membrane potentials and the unequal distribution of sodium and potassium ions between tissue and medium depend upon active transport of one or more ionic species. Since the lack of unequivocal information about intracellular concentrations and potentials in a preparation as complex as brain slices impedes determination of electrochemical potentials on either side of the membranes, it is difficult, if at all possible, to establish whether a single ion species, e.g. chloride or potassium, is in electrochemical equilibrium. As emphasized by H. H. Ussing (personal communication), the thickness of brain-cortex slices (0.25 to more than 0.5 mm) and the possible scantiness of extracellular fluid (which implies that extracellular ions to a quantitatively unknown extent may have to enter the intracellular phase en route for the incubation medium (cf. Harris & Burn, 1949) may complicate the determination of membrane-limited unidirectional fluxes by aid of isotope tracers. Measurements of influx or efflux (e.g. Levi & Ussing, 1949; Harris & Burn, 1949; Harris, 1950, 1960; Keynes, 1954; Johnson, 1955; Niedergerke, 1957; Solomon, 1960; Huxley, 1960; Winegrad & Shanes, 1962; Kleinzeller, Janáček & Knotková, 1962; Hagemeijer & van Remoortere, 1969) have nevertheless been performed both in brain slices (Krebs et al., 1951; Cummins & McIlwain, 1961; Keeseey & Wallgren, 1965; Franck & Cornette, 1966; Bourke & Tower, 1966a; Hertz, 1968; Franck, 1970, cf. also Brinley, 1963), in isolated sympathetic ganglia (Harris & McLennan, 1953; Brinley, 1967) and in the total frog brain (Zadunaisky & Curran, 1963; Bradbury, Villamil & Kleeman, 1968). The rapid inulin (and sucrose) fluxes noted in IV A, 3a indicate relatively free and fast extracellular diffusion in the slices (cf. Lund-Andersen & Hertz, 1973). The diffusion coefficients for K^+ and Na^+ are about 10 times larger (cf. Harris & Burn, 1949; Harris & McLennan, 1953), than that reported for inulin (IV A, 3a), and a halftime of 0.5-1 min may be expected for extracellular diffu-

sion of these ions from 0.5 mm thick slices provided their charges do not cause any retardation (cf. Harris & McLennan, 1953; McLennan, 1957). In the leech nervous system and the necturus optic nerve it has likewise been found that potassium ions applied to the bathing fluid diffuses rapidly in the intercellular clefts (Nicholls & Kuffler, 1964; Kuffler et al., 1966; Kuffler & Nicholls, 1966).

Potassium: All potassium in brain slices is exchangeable within about 1 hr (Fig. IV, 2), and the desaturation curves (Winegrad & Shanes, 1962) describing the washout of ^{42}K from slices previously loaded with the radioisotope indicates the presence of at least two, kinetically different compartments (Fig. IV, 3). The

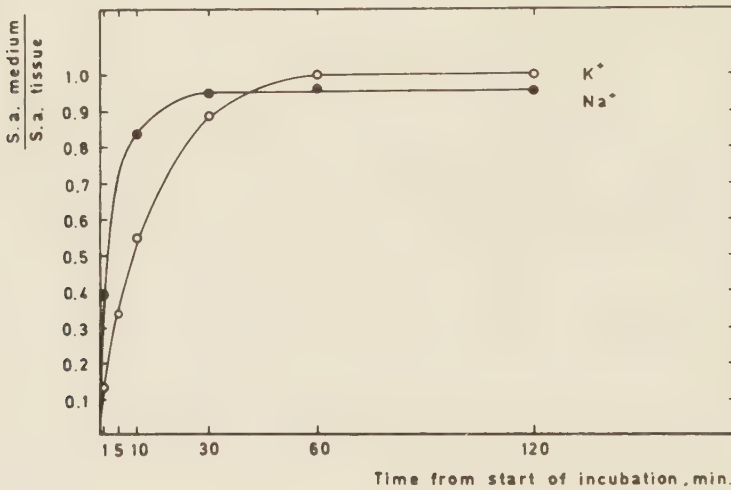


Fig. IV, 2. Uptake of ^{22}Na (●) and ^{42}K (○) into rat brain-cortex slices as a function of the time of incubation in the radioactive medium. The uptake is indicated as the ratio between the specific activity (S. a.) of the radioisotope in the medium and in the slices, i. e. 1.0 indicates a complete exchange. From Franck, 1970.

rate constant and the magnitude of the most slowly exchanging of these fractions may vary as a function of the slice thickness, but the halftime of the larger part of the rapidly exchanging compartment(s) is seen to be only slightly changed (from 12 to 15 min) when the thickness of the slice is increased from 0.25 to 1.0 mm, indicating that diffusion is not the rate-limiting process. This compartment does accordingly not represent an extracellular space and is also far larger (and contains more potassium) than the extracellular space in brain tissue (Zadunaisky & Curran, 1963; Hertz, 1968). The rapidly exchanging fraction is probably heterogeneous (cf. however the initial deviation from an exponential course described by

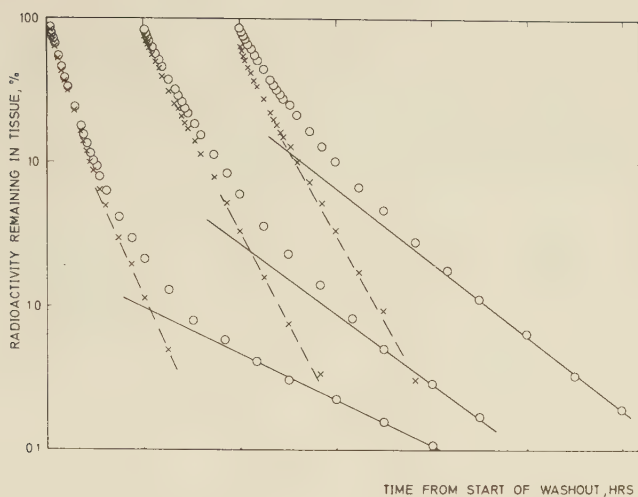


Fig. IV, 3). Washout into a non-radioactive medium of ^{42}K from rat brain-cortex slices of 0.25 (left curve), 0.5 (middle curve), and 1.0 (right curve) mm thickness, which previously had been loaded (30 min) with radioactivity (cf. Fig. IV, 2). The lines showing the washout from the two, kinetically different compartments were obtained as described in legend of Fig. IV, 1. For graphical reasons the curves have been displaced 1 hr from each other. The potassium concentration (at least in the two thinner slices) is about $70 \mu\text{moles/g}$ final wet wt. (cf. Arnfred et al., 1970).

Results are means of values obtained in two unpublished experiments by L. Lolle and L. Hertz.

e.g. Harris, 1960), and by refinement of the graphical analysis, Franck (1970) distinguished between one extracellular and two kinetically different, rapidly exchanging, cellular compartments. The slowest of these rapidly exchanging compartments, which comprises the largest amount of radioactive potassium in the tissue and is washed out with a half-time comparable to that shown in Fig. IV, 3 was envisaged by Franck (1970) to represent nerve cells and dendrites (for further details, see Franck, 1970). From the potassium concentrations and the rate constants, the fluxes between this compartment (or the tissue as a whole), and the medium can in general be estimated to between 1 and about $3 \mu\text{moles/g}$ wet wt. per min. during incubation in oxygenated, "balanced" media (Franck & Cornette, 1966; Hertz, 1968; Franck, 1970), but a slightly higher value ($6 \mu\text{moles/g}$ per min) was observed by Cummins & McIlwain (1961). Under anoxia the rate constants of the efflux from the rapidly exchanging compartment(s) are slightly increased (Hertz, 1968; Franck, 1970), but the concentration of potassium is lower and the fluxes are decreased.

Sodium: The desaturation curve observed after loading with radioactive sodium resembles in principle that obtained with potassium, since again two or more compartments are observed. These may or may not correspond anatomically to the same potassium compartments, and in this case it is the fastest of the two rapidly exchanging cellular fractions described by Franck (1970) and suggested to represent the glia cells, which accounts for most of the radioactivity. The fluxes between the rapidly exchanging cellular fraction(s) and the medium are faster than the corresponding potassium fluxes (Hertz, 1968; Franck, 1970 - cf. Fig. IV, 2), and from the rate constants of about 0.07 min^{-1} obtained in the frog brain by Zadunaisky & Curran (1963) and in rat brain-cortex slices by Hertz (1968) the efflux can be calculated to about $5 \mu\text{moles/g final wet wt.}$ (cf. also Keeseey & Wallgren, 1965). Anoxia (Hertz, 1968) or metabolic inhibitors (Zadunaisky & Curran, 1963) have been reported of little or no effect on this part of the sodium efflux, but Franck (1970) observed that the absence of oxygen slightly increased the half time for the wash-out from the rapidly exchanging cellular fraction.

The sodium exchange with the slowly exchanging fraction seems to occur at a rate of about $0.1\text{-}0.3 \mu\text{moles/g/min}$ (Zadunaisky & Curran, 1963; Keeseey & Wallgren, 1965), and is decreased by metabolic inhibitors or by a lowering of the temperature (Zadunaisky & Curran, 1963).

Chloride: Also the chloride fluxes in brain-cortex slices amount to a few micromoles per g wet weight per min (Bourke, 1969a). Both influx and efflux depend upon the chloride concentration in the medium, and the influx into the cellular compartment (Fig. IV, 4) has been shown to conform to Michaelis-Menten kinetics indicating mediated but not necessarily active transport (Bourke, 1969a).

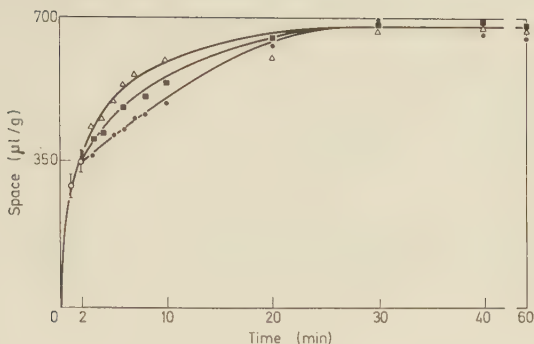


Fig. IV, 4. Fluid space (in $\mu\text{l/g}$ fresh wet wt.) accessible to ^{36}Cl in cat brain cortex slices as a function of time after addition of ^{36}Cl to the incubation medium. The slices were incubated aerobically at 37° in bicarbonate-saline-isethionate-glucose medium containing 54 mM K^+ for 30 min before the addition of $0.185 \mu\text{C}$ of ^{36}Cl to each flask. Total osmolarity of the medium was always $\sim 300 \text{ mosm/l}$, but the relative concentrations of anions differed: Δ denotes total medium chloride of 37 mM plus isethionate $\sim 100 \text{ mM}$; $\blacksquare$ denotes total medium chloride of 72 mM plus isethionate $\sim 65 \text{ mM}$; $\bullet$ denotes total medium chloride of 137 mM with no isethionate.

Results are means of values obtained in two or more experiments.

After Bourke, 1969a.

B. ION EFFECTS

1. Spaces

Application of high concentrations of potassium (Schousboe & Hertz, 1971a) or of glutamate (Cohen *et al.*, 1968; Harvey & McIlwain, 1968) may lead to a decrease in the inulin space and in the space which is accessible to sucrose added after some time of incubation (Bourke & Tower, 1966a). Almost identical spaces in "balanced" and potassium-rich or glutamate containing media were, however, reported by Franck (1970), and by Bradford & McIlwain (1966). The usual sucrose space and possibly also the thiocyanate space is virtually unaffected by glutamate or moderately high concentrations of potassium (Pappius & Elliott, 1956a; Pappius, 1965; Bourke & Tower, 1966a; Schousboe & Hertz, 1971a), and the chloride space is increased by potassium concentrations exceeding 20-30 mM (Bourke & Tower,

1966a; Bourke, 1969a; Schousboe & Hertz, 1971a; cf. also IV B, 3c). In adult rats the whole increase appears between 20 and 50 mM potassium, whereas the chloride space in new-born animals increases continuously as a function of the external potassium concentration (Schousboe & Hertz, 1971a).

2. Swelling

a. Sodium: In the total absence of electrolytes, brain slices show a great uptake of water even during incubation in media made isosmotic or hyperosmotic with sucrose or glucose. Small amounts of either potassium or sodium counteract this swelling (Elliott, 1946).

Replacement of all sodium with choline (Fig. IV, 5), tris or lithium leads to small to moderate changes in the swelling, but replacement with potassium seems to increase the swelling somewhat more (Pappius, Rosenfeld, Johnston & Elliott, 1958; Lahiri & Lajtha, 1964; Bourke & Tower, 1966a, b; Okamoto & Quastel, 1970a). In an otherwise "balanced" medium, a doubling of the sodium concentration has little effect on the uptake of water (Lund-Andersen & Hertz, 1970).

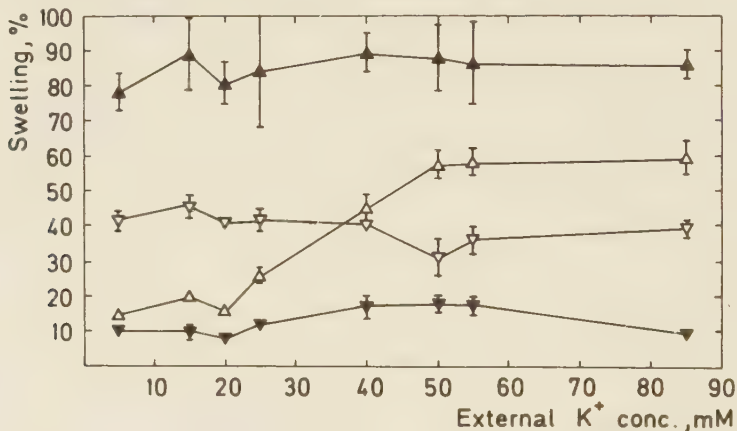


Fig. IV, 5. "Swelling" of rat brain-cortex slices as a function of the external potassium concentration after 1 hour of incubation in an otherwise "balanced", oxygenated medium (Δ); in an anoxic, "balanced" medium (▲); and in oxygenated media in which all sodium had been replaced with choline (▽) or all chloride with isethionate (▼).

Results are means of 3-25 experiments, with S. E. M. indicated by vertical bars if they extend beyond the symbols.

After Lund-Andersen & Hertz, 1970.

b. Potassium: The swelling is increased in potassium-free media (Okamoto & Quastel, 1970a) and also an augmentation of the potassium concentration above 20 mM (Fig. IV, 5) leads to a distinct increase in the degree of swelling in brain-cortex slices or the perfused cortex (Elliott, 1955; Pappius & Elliott, 1956a; Bourke & Tower, 1966a; Bourke, 1969b; Bourke et al., 1970, Bourke & Nelson, 1972). This augmentation may be competitively counteracted by an increase in the sodium concentration, but requires, on the other hand, the presence of a certain amount of sodium (Lund-Andersen & Hertz, 1970). The swelling is thus significantly greater after incubation in a sodium-containing, potassium-rich medium than after incubation in a potassium-rich medium where all sodium has been replaced with choline (Fig. IV, 5). The presence of chloride (or nitrate (L. Hertz, unpublished experiments)) is obligatory (Bourke & Tower, 1966a; Bourke, 1969b; Bourke et al., 1970; Lund-Andersen & Hertz, 1970), and no potassium-induced swelling occurs if all chloride is replaced with the indiffusible isethionate ion (cf. IV B, 2d). This may reflect the fact, that the swelling fluid appears to be an isotonic-fluid expansion composed predominantly of K^+ and Cl^- ions (Bourke, 1969b).

The potassium-induced increase in swelling of brain-cortex slices occurs both in hyperosmotic media containing an unaltered concentration of sodium (e.g. Lund-Andersen & Hertz, 1970) and in isosmotic media where the augmentation of the potassium concentration is compensated by a reduction of the sodium concentration (e.g. Bourke, 1969b). A corresponding response to excess potassium by muscle and peripheral nerve is found after incubation in isosmotic media (Boyle & Conway, 1941; Shanes, 1946; cf. also Chang et al., 1935; Tower & Bourke, 1966; Lieberman & Wright, 1966) but not after incubation in media with an unaltered sodium concentration (Boyle & Conway, 1941; Shanes, 1946; cf. Reuben, Lopez, Brandt & Grundfest, 1963). As discussed in detail by Boyle & Conway (1941) the swelling after incubation in media with reduced sodium content can both qualitatively and quantitatively be explained as a result of osmotic, electrical and Donnan equilibria, whereas a tissue containing the intracellular concentrations and charges of non-diffusible ions found in muscle cannot be expected to show any increased swelling at all, when potassium chloride is added to a medium with an unaltered sodium concentration. Provided the intracellular composition of the brain cortex does not differ drastically from that of muscle or nerve, the uptake of water under hyperosmotic conditions can accordingly not be accounted for by Donnan forces, but must be explained in another way. The observations that the potassium-induced swelling is diminished by a lowering of the temperature (Bourke, 1969b) and that the concomitant uptake of potassium and chloride (measured by aid of ^{36}Cl - cf. IV B, 5a) follows Michaelis-Menten kinetics both with respect to chloride (IV A, 3e) and with respect to potassium (Bourke, 1969a) may suggest participation of an active transport mechanism. It has therefore been suggested that the water uptake

may be secondary to an accumulation of potassium and chloride ions (Pappius, 1965; Bourke, 1969b; Lund-Andersen & Hertz, 1970; Bourke et al., 1970, Bourke & Nelson, 1972 - cf. also IV B, 3b and c). The finding that the potassium-induced swelling like the potassium-induced stimulation of oxygen uptake (II B, 2a) requires the presence of sodium but on the other hand is competitively inhibited by excess sodium is compatible with the concept of an active uptake involving an enzyme system akin to the $\text{Na}^+ - \text{K}^+$ -ATPase (cf. II B, 2a and C; VI B, 4c), and the potassium concentrations required for threshold, respectively maximum effects on swelling and on oxygen consumption are identical (Hertz, 1973; cf. Fig. IV, 5 and Fig. II, 5). An increased swelling is similarly evoked by addition of cesium in the concentrations (Fig. II, 5) required to stimulate the respiration (C. S. Kjeldsen, H. Lund-Andersen & L. Hertz, unpublished experiments).

The unaltered or decreased sucrose-, inulin- and thiocyanate spaces after incubation in potassium-rich media (IV B, 1) suggest that the swelling occurs intracellularly, and electronmicrographs have shown that almost exclusively glia cells (Zadunaisky, Wald & De Robertis, 1963; Bourke et al., 1970; Bourke & Nelson, 1972; M. Møller, H. Lund-Andersen, L. Hertz & K. Møllgård, unpublished results) are involved. This observation is supported by findings on isolated, cultivated cells (Lodin, Hartman, Kage, Korinková & Booher, 1971) and by the absence of a potassium-induced swelling in the rat until the age of about two weeks (Tower & Bourke, 1966, Franck, 1970; Schousboe & Hertz, 1971a; Schousboe, 1972).

c. Glutamate and related compounds: Also the presence of glutamate causes an increase in the degree of the swelling (Elliott, 1955; Pappius & Elliott, 1956a; Tsukada et al., 1963; Bourke & Tower, 1966a; Bradford & McIlwain, 1966; Harvey & McIlwain, 1968; Okamoto & Quastel, 1972) which again seems to occur intracellularly (cf. IV B, 1), and the effects exerted by glutamate and by potassium are not additive (Elliott, 1955; Pappius & Elliott, 1956a, cf. however also Bourke & Tower, 1966a). Some glutamate-induced swelling may be observed in the absence of chloride (Lund-Andersen & Hertz, 1970). The electron microscopic pictures presented by Tsukada et al. (1963) seem to show that the glutamate-induced water uptake may occur both into neurons and into neuroglial cells (cf. also Van Harreveld & Fifkova, 1971b). Glutamine induces a smaller swelling than glutamate (Pappius & Elliott, 1956a), and GABA causes only a very slight increase in the swelling (Tsukada et al., 1963).

d. Other anions: Total replacement of chloride with the indiffusible isethionate ion, which almost completely prevents the increased water uptake in potassium-

rich media (cf. IV B, 2b) also counteracts the increase under anaerobic conditions (Bourke & Tower, 1966a). Substitution of a bicarbonate buffer with phosphate causes similarly some decrease in the degree of the swelling (Elliott, 1955; Pappius & Elliott, 1956a; cf. also Varon & McIlwain, 1961).

e. Electrical stimulation: Prolonged application of electrical pulses may induce an increase in swelling (Thomson & McIlwain, 1961; Bachelard et al., 1962; Okamoto & Quastel, 1970a; cf. however also Varon & McIlwain, 1961).

3. Ion concentrations

a. Sodium concentration in the tissue: The sodium content in incubated tissue may be affected by changes in ionic composition of the medium or by electrical stimulation. For a comparison between results from different laboratories it is unfortunate that ion content in the tissue after incubation under different conditions has been referred either to the fresh weight, the final dry weight, the final wet weight or the supposedly intracellular volume (cf. IV A, 3c). The two former ways of expression show ion contents rather than concentrations.

Sodium concentration in the medium: A low sodium concentration in the medium leads to a reduced concentration of this ion in brain slices, and during incubation in sodium-free media the sodium level decreases to about 5 mM or less (Joanny & Hillman, 1964; Lund-Andersen & Hertz, 1970). If the sodium concentration subsequently is increased, the sodium concentration in the tissue rises to approximately the same level as in corresponding slices which have not been exposed to lack of sodium (Hillman & McIlwain, 1961; Bachelard et al., 1962; Joanny & Hillman, 1964). Oppositely, exposure to increased sodium concentrations leads to a high content of sodium in the tissue (Lund-Andersen & Hertz, 1970 - cf. Fig. IV, 6).

Potassium concentration in the medium: In the absence of potassium in the medium the sodium concentration in the tissue seems to be slightly increased (Hillman & McIlwain, 1961; Bilodeau & Elliott, 1963; Joanny & Hillman, 1964; Stahl & Swanson, 1971) from its level during incubation in media containing about 5 mM potassium.

An increase to between 16 (Bourke & Tower, 1966b) and 20 mM (Lund-Andersen & Hertz, 1970, cf. also Franck, 1970) potassium in the medium leads to a significant decrease in the sodium content (and concentration) in the tissue, whereas a further increase of the potassium concentration to between 20 and 50

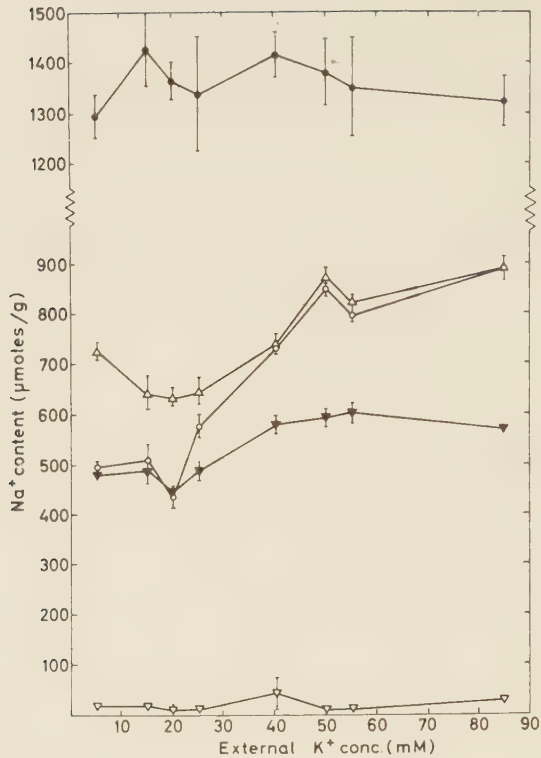


Fig. IV, 6. Sodium content ($\mu\text{moles/g}$ dry wt.) in rat brain-cortex slices as a function of the external potassium concentration after 1 hr of incubation in an otherwise "balanced", oxygenated medium (o); in an anoxic, "balanced" medium (●); in oxygenated media in which all sodium had been replaced with choline (∇) or all chloride with isethionate ($\blacktriangledown$); and in "balanced", oxygenated media to which 10 mM L-glutamate had been added (Δ).

Results are means of 3-25 experiments with S.E.M. indicated by vertical bars if they extend beyond the symbols. From Lund-Andersen & Hertz, 1970.

mM (Fig. IV, 6) causes a steep rise; this rise is attenuated, but not abolished in chloride-free media (where no increased swelling occurs - cf. Fig. IV, 5) and is accordingly not a simple consequence of the swelling (Lund-Andersen & Hertz, 1970).

Electrical stimulation: Application of electrical pulses leads to a considerable gain of sodium ions; the sodium uptake is approximately equal to a concomitant loss of potassium (Varon & McIlwain, 1961; Bachelard *et al.*, 1962; Joanny &

Hillman, 1963, 1964; cf. however also Cummins & McIlwain, 1961), and it has been suggested (McIlwain, 1963; Keeseey et al., 1965) that the increase in intracellular sodium concentration during the electrical stimulation might trigger the metabolic response (cf. however also II C). Almost identical concentrations of sodium are present in the tissue and in the medium immediately after the termination of the stimulation (cf. Fig. 5 in Bachelard et al., 1962), but the previous intracellular concentration is more or less completely regained during the following minutes (Hillman et al., 1963; Keeseey et al., 1965).

Glutamate: The uptake of potassium ions into brain slices exposed to glutamate has received much more attention than the possible changes in their sodium content. Glutamate (and certain other amino acids) have been found to increase the intracellular sodium concentration in the tissue (calculated by aid of inulin spaces), and part of the sodium uptake seems to occur almost immediately (Bradford & McIlwain, 1966; Harvey & McIlwain, 1968; McIlwain, Harvey & Rodriguez, 1969). No increase in the sodium concentration (expressed per g final wet weight) was, however, observed by Lund-Andersen & Hertz (1970) after addition of 10 mM L-glutamate, since the relatively slight increase in the content of sodium was compensated by a comparable augmentation of the swelling.

Calcium and magnesium in the medium: The absence of calcium in the medium increases the sodium content slightly, but significantly, and an increase beyond 1 mM seems to have a similar effect (Lolley, 1963; Lolley & McIlwain, 1964; Joanny & Hillman, 1964; Keeseey et al., 1965; Pull, McIlwain & Ramsay, 1970 - cf. however also Franck, 1970). The effect of isolated magnesium deficiency is doubtful, but a very high concentration of magnesium in the medium leads to an increased sodium content (Joanny & Hillman, 1964).

b. Potassium concentration in the tissue: The concentration of potassium in brain slices is influenced by the same factors as the sodium concentration.

Sodium concentration in the medium: At least 60-80 mM sodium is required in the medium to maintain a maximum concentration of potassium in brain slices (Joanny & Hillman, 1964), but sodium may to a slight extent be replaced by certain amino acids (Margolis & Lajtha, 1968). In the total absence of sodium the potassium concentration falls to 10-20 mM (Pappius et al., 1958; Takagaki et al., 1959; Hillman & McIlwain, 1961; Joanny & Hillman, 1964; Ruscak & Whittam, 1967; Lund-Andersen & Hertz, 1970), and only little reaccumulation occurs if sodium is subsequently added (Hillman & McIlwain, 1961; Bachelard et al., 1962; Joanny & Hill-

man, 1964 - cf. II B, 1a). A doubling of the sodium concentration in an otherwise "balanced" medium has only little effect on the potassium concentration (Lund-Andersen & Hertz, 1970).

Potassium concentration in the medium: In the total absence of potassium no potassium accumulation into the incubated tissue can be expected, and the potassium concentration has been found low during incubation in media containing no or only very little potassium (e.g. Pappius & Elliott, 1956b; Cummins & McIlwain, 1961; Bilodeau & Elliott, 1963; Joanny & Hillman, 1964). The possibility of potassium leakage into "potassium-free" media must be kept in mind (cf. Hillman & McIlwain, 1961; II B, 1a).

A rise in the potassium concentration from the about 5 mM found in a "balanced" medium leads to an increased potassium concentration in the tissue. The rise in the concentration is steep in the interval 5-20 mM (Fig. IV, 7), but negligible in the range 20(25)-50 mM (Lund-Andersen & Hertz, 1970; Franck, 1970). The high potassium concentration at an external potassium concentration around 20 mM is mirrored by a low sodium concentration (IV B, 3a) indicating a, probably active, exchange between the two ions. It is in keeping with this concept that the swelling is unaffected (IV B, 2b). The interval 20 (25) to 50 mM external potassium, in which the difference between the internal and external potassium concentration is reduced, is in contrast the same level which causes an increase in the swelling (cf. Fig. IV, 5). That the relative reduction of the internal potassium concentration is no direct consequence of the swelling is seen from the fact that the potassium concentration is affected in exactly the same manner by the external potassium concentration (Fig. IV, 7) after incubation in chloride-free or glutamate-containing media in which the swelling is not influenced by the external potassium concentration (Fig. IV, 5). Accordingly, the content of potassium per g dry weight (or g initial wet weight) is also relatively constant between 20 (25) and 50 mM potassium in the chloride-free and the glutamate-containing medium, whereas it shows a continuous rise in the potassium-rich, otherwise "balanced", medium (Lund-Andersen & Hertz, 1970; cf. also Pappius & Elliott, 1956b). These findings led Lund-Andersen & Hertz (1970) to suggest the hypothesis (Fig. IV, 8) that the depolarization evoked by the increase of the external potassium concentration (Fig. IV, 9) in any case leads to a potassium loss and a sodium gain, and that the potassium loss in the "normal" medium may be balanced by a compensatory accumulation of potassium and chloride (cf. Bourke, 1969a, b), which for osmotical reasons leads to an increased swelling (IV B, 2b). No corresponding extrusion of sodium seems to occur (IV B, 5a; cf. also IV C).

Under anoxic conditions or in the absence of sodium the potassium concentration rises rectilinearly as a function of the external potassium concentration

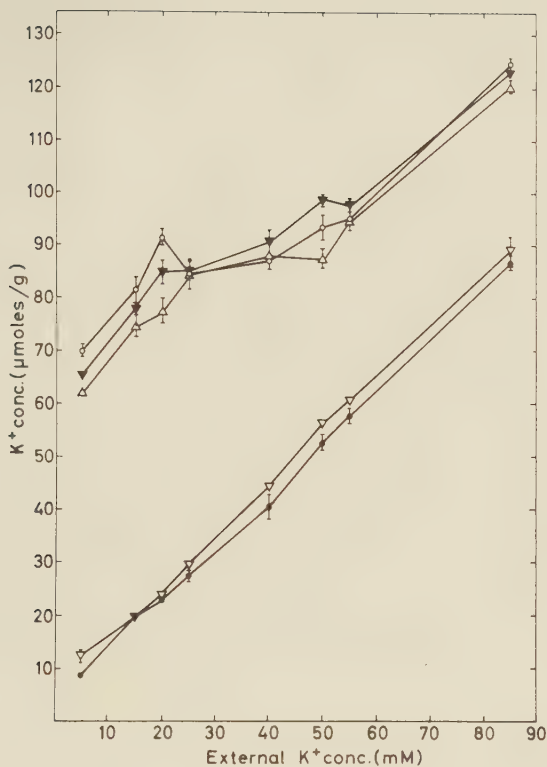


Fig. IV, 7. Potassium concentration ($\mu\text{moles/g}$ final wet wt.) in rat brain-cortex slices as a function of the external potassium concentration after 1 hour of incubation in an otherwise "balanced", oxygenated medium (o); in an anoxic "balanced" medium (●); in oxygenated media in which all sodium had been replaced with choline (∇) or all chloride with isethionate ($\blacktriangledown$); and in "balanced", oxygenated media to which 10 mM L-glutamate had been added (Δ).

Results are means of 3-25 experiments, with S. E. M. indicated by vertical bars if they extend beyond the symbols. From Lund-Andersen & Hertz, 1970.

(Fig. IV, 7). The slope of the rise is 0.9 corresponding to a water content of about 9/10 in these, grossly swollen, slices.

It is in accordance with the suggested neuroglial localization of the postulated potassium-induced uptake of potassium ions (cf. II B, 2c, III B, 2a, IV B, 2b) that a relatively great accumulation of potassium is observed in isolated glial cells obtained by centrifugation and incubated in potassium-rich media, whereas the corresponding nerve cell fraction shows only little potassium accumulation

(Henn, Haljamäe & Hamberger, 1972). Based upon this observation and the high Na^+ - K^+ -ATPase activity in glia cells (VI B, 4c), Hamberger and coworkers (Haljamäe & Hamberger, 1971; Henn *et al.*, 1972) suggested an active uptake of potassium ions mediated by the Na^+ - K^+ -ATPase and occurring specifically into glia cells (cf. IV C).

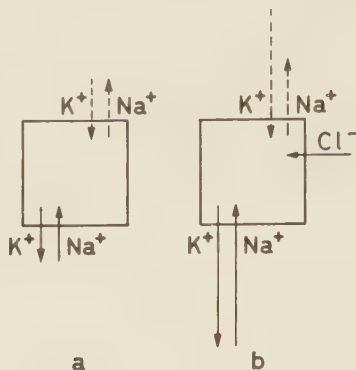


Fig. IV, 8. Diagrammatic representation of movements of potassium and sodium ions in brain slices exposed to 5 or 50 mM external potassium. The solid-line arrows indicate the net uptakes or releases which would occur if only diffusion took place, whereas the dashed arrows show movements which probably are evoked by active transport. Each square represents the same amount (dry weight) of tissue.

In the medium with 5 mM K^+ (Fig. a) the active accumulation of potassium equals the potassium loss by diffusion, and the active extrusion of sodium equals the sodium uptake by diffusion. Accordingly the potassium content is kept constant above, and the sodium content below the medium concentrations. No swelling occurs.

An increase of the external K^+ concentration to 50 mM (Fig. b) causes a large rise of the permeability to both sodium and potassium. Simultaneously the active uptake of potassium ions is enhanced without any corresponding increase of sodium extrusion. Accordingly the net loss of potassium becomes smaller than the net gain of sodium. A net uptake of negatively charged ions, probably chloride must occur together with the uptake of potassium ions. This leads to a cellular hyperosmolarity and thus to an increased swelling.

After Lund-Andersen & Hertz, 1970

Calcium and magnesium in the medium: The presence of calcium is essential in order to obtain a maximum concentration of potassium in the tissue (Gardos, 1960), 1961; Lolley & McIlwain, 1964; Joanny & Hillman, 1964; Keeseey *et al.*, 1965; Franck, 1970; cf. however also Ruscak & Whittam, 1967). Also an increase in

the calcium concentration beyond 1 mM may cause some lowering of the potassium content (Keeseey et al., 1965; cf. however also Franck, 1970). A suitable concentration of magnesium may replace calcium in securing a maximum potassium content in the tissue (Gardos, 1960; but the role of this ion seems less evident (Joanny & Hillman, 1964).

Glutamate: The simultaneous presence of glutamate and glucose in the medium leads to a specially high content of potassium in brain slices and in isolated retinæ, which is due to a pronounced re-accumulation of potassium ions at the start of the incubation (Turner et al., 1950). Also glutamate ions are taken up against a concentration gradient (cf. V A, 2b), and a coupled transport of these ions has been suggested (Turner et al., 1950; Tsukada et al., 1963; Hertz, 1968). Takagaki et al. (1959) observed, however, no strict correlation between the accumulation of glutamate and of potassium, and the fluxes of potassium and of glutamate between the tissue and the incubation medium differ from each other by orders of magnitude (Arnfred & Hertz, 1971).

The increased potassium content in glutamate-containing media has been confirmed by several authors (e.g. Elliott, 1955; Pappius & Elliott, 1956b; Bilodeau & Elliott, 1963; Joanny, Hillman & Corriol, 1966), but the effect may be variable and is often only small (Weil-Malherbe, 1960; Bradford & McIlwain, 1966; Lund-Andersen & Hertz, 1970). The uptake occurs relatively slowly (Pappius & Elliott, 1956b; Bradford & McIlwain, 1966; Harvey & McIlwain, 1968; Lund-Andersen & Hertz, 1970), and it is correlated with an increase in swelling (IV B, 2c) of the non-inulin space (IV B, 1). This means that the potassium concentrations in the cells rather show a decrease (Pappius & Elliott, 1956b; Harvey & McIlwain, 1968; Lund-Andersen & Hertz, 1970). The increase in potassium content is preceded by a small, but significant, loss of potassium immediately after the addition of glutamate (Harvey & McIlwain, 1968; Lund-Andersen & Hertz, 1970), and it may be accompanied by an increase in the sodium content (IV B, 3a). The latter ionic changes are probably secondary to the glutamate-induced depolarization (IV B, 4c). The uptake of potassium (and water) has been suggested to be induced by an increased intracellular sodium concentration (Harvey & McIlwain, 1968), but could as well result from an increased extracellular potassium concentration (cf. Lund-Andersen & Hertz, 1970). D-glutamate is somewhat less effective than L-glutamate in securing a high potassium content (Takagaki et al., 1959; Tsukada et al., 1963 - cf. II B, 2a and IV B, 4c). Aspartate (Turner et al., 1950; Takagaki, 1963; Tsukada et al., 1963), which also may evoke a depolarization (cf. IV B, 4c), has a similar effect.

The simultaneous presence of glutamate and high concentrations of potassium (and glucose) has been reported to yield a very high concentration of potassium

(Takagaki et al., 1959), but this finding could not be reproduced by Lund-Ander-
sen & Hertz (1970). The considerable reduction in potassium content evoked by
complete lack of sodium seems to be less pronounced when both glucose and glu-
tamate are present in the medium (Takagaki et al., 1959).

Electrical stimulation: Application of electrical pulses causes a fall in the pot-
assium concentration of brain slices; the decline begins immediately, is inde-
pendent of the substrate used (cf. VI B, 2a-e) and reaches a maximum of about
20 μ equiv./g wet wt. after 5-10 min (Cummins & McIlwain, 1961; Joanny & Hill-
man, 1964). Subsequently the potassium concentration remains stable at a level
which depends upon the sodium concentration in the medium (Bachelard et al.,
1962), and the potassium concentration in the extracellular clefts may conceivably
increase as much as 20-30 mM (Hillman & McIlwain, 1961; cf. also IV C). During
the first 2-3 minutes after the termination of the stimulation an almost quantitive
re-accumulation of potassium occurs (Keesey et al., 1965).

Drugs: Addition of labilizers causes a decrease of the potassium concentration
in the tissue (Wollenberger, 1955a; Yoshida, Kaniike & Namba, 1963).

The potassium loss evoked by electrical stimulation occurs even in the pre-
sence of dinitrophenol, but no reaccumulation takes place (McIlwain et al., 1961).
Chlorpromazine (Hillman et al., 1963) and tetrodotoxin (McIlwain, 1967) almost
completely prevent both the potassium loss and the sodium gain during electrical
stimulation, but the increased sodium uptake in the presence of excess potassium
is unaffected by tetrodotoxin (Okamoto & Quastel, 1970b). Protamine reduces the
potassium loss and the sodium gain during the stimulation slightly, but the reac-
cumulation of potassium and extrusion of sodium is almost completely inhibited
(McIlwain et al., 1961; Hillman et al., 1963; cf. also II B, 4 and II C).

c. Chloride concentration in the tissue: Addition of excess potassium as the
chloride salt causes an increase of the chloride concentration in the tissue, and
also the ratio between the chloride concentration in the tissue (expressed per g
final wet weight) and that in the medium, i. e. the chloride space, may be con-
siderably larger in potassium-rich than in "balanced" media (cf. IV B, 1). Such
an increase is to be expected if a depolarization is evoked and the chloride ion is
in electrochemical equilibrium (cf. table IV, 1), but stimulation of an active trans-
port mechanism has also been suggested (IV B, 2b). If the oxygenation of the me-
dium is not optimum (cf. Arnfred et al., 1970; Franck, 1970) the chloride concen-
tration may be high already after incubation in a "balanced" medium, and no fur-
ther increase is evoked by excess potassium (Hertz, 1968). Application of elec-

trical pulses or addition of glutamate have been reported to evoke little change or even a decline in the total chloride content of the tissue (Varon & McIlwain, 1961; Cummins & McIlwain, 1961; Bourke & Tower, 1966b), but an increased concentration in the non-inulin space was found by Keesey et al. (1965).

Replacement of sodium in the medium with choline leads to an increase in the chloride concentration (Bourke & Tower, 1966a), but 30 mM sodium is sufficient to maintain a "normal" chloride level (table 3 in Bachelard et al., 1962).

d. Calcium concentration in the tissue: The calcium concentration in incubated brain slices depends upon the calcium concentration in the medium but is generally somewhat higher (Lolley, 1963). Application of electrical pulses (Huttunen, 1969; cf. however also Lolley, 1963) or addition of L-glutamate (Ramsay & McIlwain, 1970), excess calcium (Huttunen, 1969) or excess potassium may cause an increase in the calcium content, whereas an increase of the external magnesium concentration leads to a decrease (Huttunen, 1969).

4. Membrane potential

a. Potassium: Hillman & McIlwain (1961) observed no membrane potentials in brain slices incubated in media prepared without potassium. This was in spite of a potassium concentration of 44 μ equiv./g initial wet weight in the tissue and about 1 mM in the medium (due to leakage from the cells) in their experiments. A slight increase in the external potassium concentration was found to lead to the appearance of membrane potentials, and with an external concentration of 6.2 mM potassium the potentials reach their maximum value (-59 mV). A further increase of the external potassium concentration causes a decrease in the magnitude of the potentials and at 50 mM the membrane potentials are abolished (cf. Fig. IV, 9). They may, however, reappear if the tissue subsequently is exposed to a lower potassium concentration (Hillman & McIlwain, 1961; Gibson & McIlwain, 1965). Essentially similar results have been obtained (at 23⁰) with isolated neurons from Deiters' nucleus (Hillman & Hydén, 1965a). The maximum potentials were, however, lower (-49 mV), and small membrane potentials (i.e. less than -30 mV) were obtained both in the potassium-deficient and in the potassium-rich media.

The membrane potential in mammalian glial cell cultures (Hild et al., 1958; Hild, 1964; Wardell, 1966) or in the leech packet glial cell (Nicholls & Kuffler, 1964) and necturus optic nerve (Kuffler et al., 1966) is analogously abolished by high external concentrations of potassium. In the latter preparations the contained glia cells behave as perfect potassium electrodes even when the external

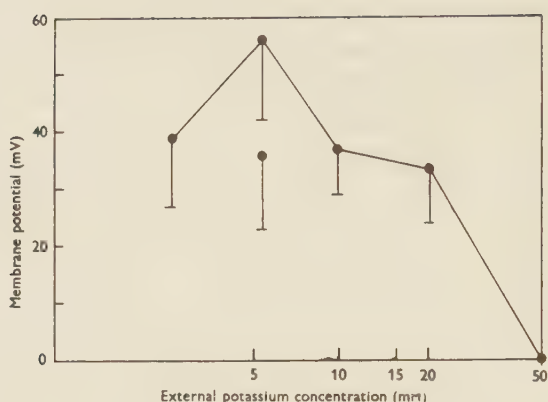


Fig. IV, 9. Resting potentials in a single guinea-pig brain-cortex slice as a function of the potassium concentration (shown logarithmically) in an incubation medium with an unaltered sodium concentration. Each circle gives the mean of 25 readings, with the S.D. represented by a vertical line. When the tissue had been depolarized for 100 min, it was returned to 6.2 mM KCl, and the lower circle at 6.2 mM KCl shows the potentials recorded at that concentration after repolarization. After Hillman & McIlwain, 1961.

potassium concentration is lowered to 1.5 mM (Nicholls & Kuffler, 1964; Kuffler *et al.*, 1966; Kuffler & Nicholls, 1966; Kuffler, 1967; cf. also Dennis & Gerschenfeld, 1969). Stimulation of neurons may lead to a depolarization of adjacent glial cells. This is probably due to the release of potassium ions as shown by Kuffler and coworkers in a series of elegant experiments in which the external potassium concentration was varied between 1.5 and 4.5 mM, and the observed potential change after identical degree of stimulation, i.e. the same release of potassium, was found to vary in accordance with the Nernst equation (Orkand, Nicholls & Kuffler, 1966; Kuffler & Nicholls, 1966; cf. Fig. IV, 10). A neuroglial depolarization in response to neuronal activation (Grossmann & Hampton, 1968; Grossman *et al.*, 1969) or application of excess potassium (Krnjević & Schwartz, 1967) has also been observed in unresponsive ("idle") cells, i.e. glia cells (Kelly, Krnjević & Yim, 1967; Grossman & Hampton, 1968) of the mammalian brain cortex in vivo. These cells do not behave as perfect potassium electrodes (Pape & Katzman, 1970, 1972), and calculation of intracellular potassium and chloride concentrations in brain-cortex slices shows that both potassium and chloride cannot be in electrochemical equilibrium (Table IV, 1; cf. also Allen, 1955).

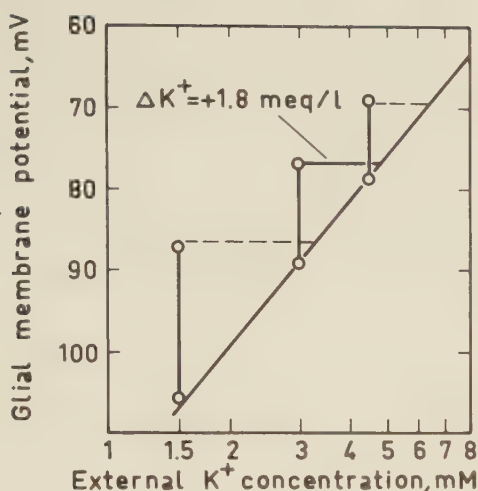


Fig. IV, 10. Test for the liberation of K^+ by nerve impulses as the cause of glial depolarization. All measurements were made on one glial cell in the optic nerve of necturus. The oblique line is the theoretical K^+ concentration-glial membrane potential curve predicted by the Nernst equation with a slope of 59 mV for a 10-fold change in K_o at 18° . The circles on this line indicate the glial resting potential at the three concentrations of external K^+ , 1.5, 3.0 and 4.5 mM. At the normal resting potential (89 mV) in Ringer solution (3.0 meq/liter K^+) the optic nerve was stimulated by a train of impulses which caused a glial depolarization of 12.1 mV (middle upper circle). From the curve it can be seen that this depolarization is equivalent to the addition of 1.8 meq/liter K^+ to the bathing fluid. If this is the amount of K^+ liberated by the train of nerve impulses in Ringer solution one can predict from the curve the size of glial depolarizations which will be set up by the same train of impulses when the K^+ content in the bath is lower or higher. With 1.5 meq/liter of K^+ in the bath the same train of nerve impulses depolarized the glial cell by 18.5 mV (lower circle on the line); the predicted effect of adding 1.8 meq/liter K^+ to 1.5 meq/liter was almost the same (lower dotted line). Similar good agreement between predicted and observed values is seen when the bath contained 4.5 meq/liter K^+ . From Orkand et al., 1966.

The glia cell membrane reacts to potential changes without any "all-or none" action potential (Hild et al., 1958; Hild & Tasaki, 1962; Kuffler & Potter, 1964; Kuffler et al., 1966; Kuffler & Nicholls, 1966; Wardell, 1966; cf. also Dennis & Gerschenfeld, 1969), but potential changes may spread between individual glial cells over a distance of 0.2 to more than 1 mm (Kuffler & Potter, 1964; Kuffler

et al., 1966; Kuffler & Nicholls, 1966; Walker & Hild, 1969). The electrical contacts between the individual cells probably occur at specialized "junctions" (Gray, 1961; Peters, 1962; Coggeshall & Fawcett, 1964; Brightman & Reese, 1969). Provided the glia cells behave as potassium electrodes, the potassium ions released during nervous activity may enter the joined cells passively in one region, traverse the cells and leave in another region, i. e. such glia cells may function as a passive "spatial buffer" which removes potassium ions from the immediate extraneuronal environment (Orkand et al., 1966; Kuffler & Nicholls, 1966; Kuffler, 1967; Trachtenberg & Pollen, 1970; Orkand, 1970; Trachtenberg, Kornblith & Häuptli, 1972 - cf. also IV C).

b. Electrical stimulation: Application of electrical pulses for 1 to 10 min causes a considerable decrease of the membrane potential in brain-cortex slices (Hillman et al., 1963). The presence of chlorpromazine or protamine has little or no effect on the depolarization and subsequent repolarization (Hillman et al., 1963). Postsynaptic potentials (Yamamoto & McIlwain, 1966a, b; Richards & Sercombe, 1968), and provoked spike potentials (Richards & McIlwain, 1967) have been obtained in slices from the anterior piriform lobe, which may be stimulated directly or via the lateral olfactory tract. Postsynaptic potentials may also be recorded in slices from the superior colliculus (Kawai & Yamamoto, 1969) and the hippocampus (Yamamoto & Kawai, 1967) of the guinea-pig. The potentials are very sensitive to the reduction in the ATP level produced by relative hypoxia (Richards & Sercombe, 1968; Yamamoto & Kurokawa, 1970), and to deviations in the concentrations of sodium and potassium (Miura, 1970).

c. Glutamate and related compounds: Addition of L-glutamate leads to a prompt decrease of 15-30 mV in the numerical magnitude of the resting membrane potential in brain-cortex slices (Hillman & McIlwain, 1961; Gibson & McIlwain, 1965; Bradford & McIlwain, 1966). The change is reversible unless ammonium is added simultaneously. Addition of this ion alone has no effect, and D-glutamate does also not affect the resting membrane potential (Hillman & McIlwain, 1961). Certain other amino acids (i. e. L-aspartate, L-cysteate, D, L-homocysteate and L- α -aminoadipate) cause also a depolarization, whereas β -alanine and glutamate are without effect (Bradford & McIlwain, 1966).

The "unresponsive", i. e. glial (Kelly et al., 1967; Grossman & Hampton, 1968) cells in the cat brain cortex react to extracellular application of GABA with a depolarization. Glutamate (which leads to a neuronal depolarization) has no effect on the membrane potential of glia cells in the living animal (Krnjević & Schwartz, 1967) or in culture (Wardell, 1966).

d. Sodium: The resting membrane potentials in brain slices depend upon the presence of sodium (Hillman & McIlwain, 1961). This is probably related to the inhibition of potassium accumulation in sodium-free media. It is in accordance with this point of view that subsequent addition of sodium neither leads to a re-establishment of the potassium content (cf. IV B, 3b) nor causes a repolarization (Hillman & McIlwain, 1961). As little as 26 mM sodium in the medium is sufficient to maintain maximum polarization, and an increase of the sodium concentration to 300 mM does also not affect the resting membrane potentials (Hillman & McIlwain, 1961; Gibson & McIlwain, 1965). The level of the sodium concentration in the medium which is compatible with the presence of high membrane potentials in brain slices is thus extremely broad.

In the isolated neurons from the Deiters' nucleus relatively high (-25 to -30 mV) membrane potentials have been observed in the total absence of sodium. Maximum polarization occurs with a sodium concentration about 110 mM, and a further increase causes some decline (Hillman & Hydén, 1965a).

e. Calcium and magnesium: The absence of either calcium or magnesium from the medium leads to a delayed appearance of the potentials in brain-cortex slices at the start of the incubation. When polarization finally occurs, it is of almost normal size and may conceivably be caused by traces of these ions which have leaked out from the tissue (Hillman & McIlwain, 1961). The postsynaptic potentials are abolished in calcium free media (Miura, 1970).

The membrane potentials seem to be more stable during incubation in a medium containing 2.6 mM calcium instead of 0.75 mM (Gibson & McIlwain, 1965). Extremely high concentrations (14-20 mM) of either calcium or magnesium are without effect on the resting potentials (Hillman & McIlwain, 1961).

5. Ion transport

a. Potassium effects: The difficulties encountered in the study of ion transport in brain slices (IV A, 3e) become even greater if the ion concentrations in the medium are altered. The effects by excess potassium on the kinetics of potassium exchange seem thus not to be quite the same when the high potassium concentrations are present from the start of the incubation and when they are added during the washout (Franck, 1970). Furthermore, H. H. Ussing (personal communication) has pointed out that even an extracellular potassium compartment may show a potassium-induced stimulation of the efflux, if the extracellular diffusion occurs so slowly that the ions to a considerable extent enter cellular com-

partments on their way to the incubation medium (IV A, 3e), and if the latter process shows saturation kinetics. The possible paucity of extracellular fluid in brain (cf. I B, 2) indicates that this source of error might be of importance in brain slices, but the rapid inulin flux (IV A, 3a) shows, on the other hand, that extracellular diffusion occurs so fast compared with the exchange across cell membranes in the slices, that the cellular re-accumulation will be small.

Potassium fluxes: Addition of 50 mM potassium during the wash-out causes a considerable increase in the potassium efflux (Fig. IV, 11) and influx in the rapidly exchanging cellular (IV A, 3e) fraction(s) (Hertz, 1968; Franck, 1970). Also the potassium concentration in the tissue increases and the magnitude of the fluxes seem to increase from 1-2 $\mu\text{moles/g}$ final wet weight per minute in the "balanced" medium (cf. IV A, 3e) to about 10 $\mu\text{moles/g}$ after the potassium addition.

This value is remarkably close to the net uptake after termination of electrical stimulation (IV B, 5c). Almost the same increase in the rate constant of the wash-out is evoked by addition of 20 mM potassium (Franck, 1970; L. Hertz, unpublished experiments). This is less than the potassium concentration required to exert a marked effect on oxygen uptake (II B, 2a - Fig. II, 5), and swelling (IV B, 2b - Fig. IV, 5), and the potassium-induced increase of ion fluxes may, in contrast to the effect on the two other phenomena, be observed in slices from new-born rats (Schousboe & Hertz, 1971b). Addition of excess potassium after 2-3 hours of washout, i. e. at a time when the shape of the curve is predominantly determined by the slowly exchanging component, has a much smaller effect (Hertz 1968; Franck, 1970). If the high concentrations of potassium are present during the whole preincubation and wash-out, the increase in the rate constants of the three cellular fractions envisaged by Franck (1970) is less drastic, but the rapidly exchanging, second fraction which was interpreted to represent glia cells, becomes much larger. During incubation under anoxic conditions the increase in the potassium concentration is of at least the same magnitude (cf. Fig. IV, 7), but the rate constant increases only slightly after potassium addition, and the increase in influx and efflux becomes much smaller, i. e. from about 1 to about 4 $\mu\text{moles/g}$ final wet weight/min (Hertz, 1968; cf. also Franck, 1970).

Sodium fluxes: The curve describing the influx of sodium into brain-cortex slices (Fig. IV, 2) seems to be independent of an increase in the external potassium concentration (Hertz, 1968; Franck, 1970), but it should be remembered that the tissue is not in a steady state since the sodium content increases (IV B, 3a). The rate constant of the efflux from the rapidly exchanging fraction (Hertz, 1968), or at least from its quantitatively dominant, possibly neuroglial, second compartment

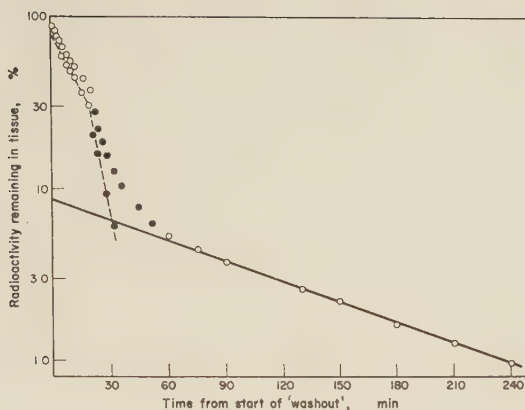


Fig. IV, 11. Wash-out into a non-radioactive medium of ^{42}K from rat brain slices previously "loaded" with radioactivity. The preincubation in the radioactive medium (30 min) and the greater part of the wash-out (represented by o-o) were performed in a medium containing 5 mM potassium; during the period 20-52 min the potassium concentration in the medium was increased by 50 mM (represented by ●-●). The air phase was O_2/CO_2 .

The fully drawn and the dotted line were obtained as described in the legend of Fig. IV, 1.

Results are means of values obtained in 5 experiments. From Hertz, 1968.

(Franck, 1970), is also not affected by excess potassium. In the relatively small, third compartment described by Franck (1970) high potassium concentrations have, however, been found to induce a transitory increase.

The rate of potassium efflux from muscle is also enhanced by a high concentration of potassium (L. Hertz, unpublished experiments) or application of electrical pulses (Weiss, Coalson & Hurwitz, 1961; Hagemeijer, 1968), but the influx of sodium shows a concomitant increase (Hodgkin & Horowicz, 1959; Kao, Bronner & Zakim, 1960; Nagasawa, 1964). This is also the case in giant-fibre preparations from non-mammalian nerve (Keynes, 1951; Hodgkin & Keynes, 1955). No increased sodium influx could, however, be demonstrated in the rabbit vagus nerve, conceivably because the bulk of the ion exchange occurred with non-axoplasmatic tissue, e.g. the Schwann cells (Keynes & Ritchie, 1965).

Calcium fluxes: The influx of calcium ions into muscle is increased by excess potassium. This may lead to an augmentation of the concentration of free calcium ions in the cell interior and thus to a rise of calcium efflux (Bianchi & Shanes, 1959; Shanes & Bianchi, 1960; Winegrad & Shanes, 1962). This response is en-

hanced when nitrate replaces chloride in the medium (Bianchi & Shanes, 1959), which may explain the increased metabolism (II B, 4), and it is abolished by drugs which counteract the stimulation of oxygen uptake without major effect on the depolarization (Novotný & Vyskocil, 1965). The increase in intracellular calcium concentration may lie behind the potassium induced (or calcium induced) (II B, 2a) augmentation of oxygen consumption and lactate production in muscle (Novotný *et al.*, 1962; Novotný & Vyskocil, 1963; Novotný, 1965; Novotný & Vyskocil, 1965), and it may play a crucial role in the linkage between muscular excitation and contraction (e.g. Niedergerke, 1956a, b; Shanes, 1958b; Bianchi & Shanes, 1959; Bianchi, 1968). Analogously the uptake of calcium ions into peripheral nerve and ganglia is increased by electrical stimulation or by high concentrations of potassium (Hodgkin & Keynes, 1957), and the calcium uptake may be related to the potassium-induced release of acetylcholine (V B, 1g). Uptake of calcium in mechanically damaged parts of the preparation may complicate work on calcium fluxes in brain slices (cf. Lipicky, Hertz & Shanes, 1963). Both calcium influx and efflux seem, however, to be transitorily increased by electrical stimulation of brain slices (Lolley, 1963), whereas addition of glutamate was found only to enhance the influx (Ramsay & McIlwain, 1970) and thus lead to an increased calcium content (IV B, 3d).

Chloride fluxes: Addition of excess potassium during the wash-out of ^{36}Cl from brain-cortex slices has no effect on the rate constant (Hertz, 1968; Schousboe & Hertz, 1971a), but the chloride content is increased. The initial velocity of the chloride influx into brain-cortex slices or cultivated astrocytes is significantly increased by excess potassium (Bourke, 1969a; Gill, Young & Tower, 1963; cf. IV B, 2b).

b. Sodium effects: Addition of sodium chloride during the wash-out causes a slight increase in the rate constant of potassium efflux (L. Hertz, unpublished experiments), but the change is considerably smaller (from 0.041 to 0.053 min^{-1}) than that observed after addition of potassium. It is of the same magnitude under anoxia, and may conceivably be evoked by solvent drag as a result of the increased tonicity of the medium.

c. Electrical stimulation: Application of electrical pulses causes an unequivocal increase of the potassium efflux but the effect on sodium transport seems more ambiguous (Cummins & McIlwain, 1961; Keeseey & Wallgren, 1965; Franck, 1970).

Potassium fluxes: During application of electrical pulses, both in- and efflux of potassium increase to about 10 $\mu\text{moles/g wet wt. /min}$ (Cummins & McIlwain, 1961; cf. also Franck & Cornette, 1966; Franck, 1970), i.e. approximately the

same value as after addition of high concentrations of potassium. Some effect is also exerted on the slowly exchanging fraction (Franck, 1970). The raised influx continues during the first minutes after the termination of the electrical stimulation and leads to a considerable ($4\text{--}10\ \mu\text{moles/g wet wt. /min}$) net gain of potassium (Cummins & McIlwain, 1961; Bachelard *et al.*, 1962; Keeseey *et al.*, 1965).

Sodium fluxes: It has been claimed by Keeseey & Wallgren (1965) that both influx and efflux of sodium are raised by application of electrical pulses, but Franck (1970) found the stimulation to have little effect on the efflux of ^{24}Na . The wash-out curves obtained by Keeseey & Wallgren (1965) in the presence and in the absence of electrical stimulation are shown in Fig. IV, 12. It can be seen that the radioactivity corresponding to the first point measured during the efflux is slightly (but insignificantly) lower in the experiments in which pulses were applied. Subsequently, i. e. from 1 to 10 minutes, the desaturation curves follow almost completely identical courses whether pulses are applied or not. Since it is the slope of the curve that indicates the rate constant and thus the efflux, application of electrical pulses has apparently no effect on the efflux of ^{22}Na .

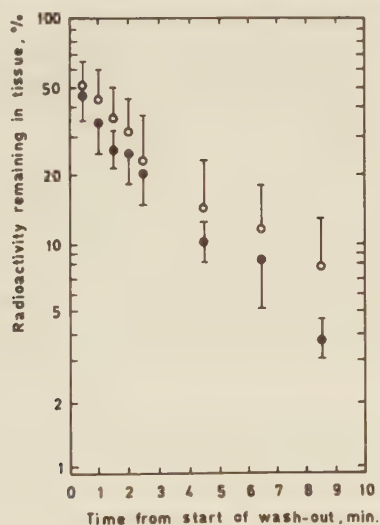


Fig. IV, 12. Wash-out into a non-radioactive medium of ^{24}Na from guinea pig brain-cortex slices previously loaded with radioactivity.

The upper curve (o) shows the wash-out into a "balanced" medium without application of electrical pulses, whereas the lower curve (●) shows the efflux into a similar medium from electrically stimulated slices in the steady state. The vertical bars indicate standard deviations.

Results are means of 7 (o) and of 3 (●) experiments. From Keeseey & Wallgren, 1965.

The augmentation of the influx during the electrical stimulation seems better established (Keesey & Wallgren, 1965). It is also consistent with the relatively large increase in sodium content evoked by electrical stimulation (cf. IV B, 3a) and an unchanged efflux that the rate of sodium uptake at least must be temporarily increased.

d. Glutamate effects: L-glutamate resembles high concentrations of potassium and electrical stimulation in causing an increase in oxygen uptake (II B, 2a), aerobic lactate production (VI B, 3b) and utilization of energy-rich phosphates (III B, 6a) together with a decrease of membrane potential (IV B, 4c). Addition of L-glutamate causes, however, no increase in the efflux of ^{42}K from brain-cortex slices (L. Hertz, unpublished experiments), but an increase in the sodium influx into the intracellular compartment has been reported (Harvey & McIlwain, 1968). GABA/evokes, in contrast, a slight, transient increase in potassium efflux after topical application to the exposed rabbit cortex (Brinley *et al.*, 1960).

C. DISCUSSION

Ion content and membrane potentials must be closely interconnected. Nevertheless no membrane potentials could be recorded from slices incubated in "potassium-free" media (containing 1 mM potassium), though the potassium concentration in the tissue was $44\text{ }\mu\text{moles/g}$ initial wet wt., and the gradient between internal and external potassium concentrations accordingly consistent with considerable polarization. Furthermore, chlorpromazine and protamine were found to affect the changes in potassium concentration during or after electrical stimulation with little effect on membrane polarization. These findings emphasize that the slices cannot be regarded as consisting of only two phases, i. e. an extracellular compartment, which contains little potassium, and a cellular compartment which contains almost all the potassium ions of the tissue and from which the potentials are recorded. Also the desaturation curves seem to show that the ion exchange occurs corresponding to several different compartments. This implies that any calculation of the actual concentrations of ions in the different cell types in the brain is encumbered with considerable uncertainty.

Difficulties are also encountered in distinguishing between active and passive transport mechanisms, though it seems beyond doubt that an active transport is involved in keeping the potassium concentration higher and the sodium concentration lower in the tissue than in the surroundings. Under poor metabolic conditions (anoxia or sodium lack) the tissue potassium was found to show a passive rise in parallel with the potassium concentration in the medium. The slope (0.9) cor-

responds to the water content, suggesting identical activity coefficients in the incubation medium and in the cells (cf. also Boyle & Conway, 1941). The net loss of potassium ion, the gain in sodium, and the increased potassium efflux during the application of electrical pulses (and initially after addition of glutamate) might be due to an increased cell permeability evoked by the depolarization, since potassium ions move from a higher to a lower concentration, and since the loss occurs independently of the substrate used (cf. VI B, 2a-e). Such a release of potassium from cellular sources may lead to an increase in the extracellular potassium concentration (cf. Hillman & McIlwain, 1961; Orkand et al., 1966; Grossman et al., 1969; see also IV B, 3b and IV B, 4a), and either the increased intracellular sodium concentration (McIlwain, 1963; Bradford & McIlwain, 1966; Chan & Quastel, 1967, 1970) or the increased extracellular potassium concentration may lie behind the metabolic effects exerted by "electrical stimulation" or addition of glutamate (cf. II C). These effects probably include an increased active uptake of potassium ions which continues beyond the actual period of stimulation and leads to a net uptake after the termination of the pulses. The stimulation of potassium efflux during incubation in potassium-rich media may analogously result from a depolarization and a consequent increase in the membrane permeability. Again the influx is increased (Franck, 1970), but potassium effects on potassium influx are difficult to interpret since the great augmentation in the external potassium concentration per se must lead to a considerable increase in the diffusion rate. The differences in influx between oxygenated and anoxic slices (Hertz, 1968) suggest, however, that a considerable part of the increase in influx after exposure to 50 mM potassium is dependent upon energy metabolism. Such a comparison between oxygenated and anoxic tissue is weakened by the possibility of changes in membrane permeability or extracellular space, but the inulin space is unaffected by anoxia (IV A, 3b), and no indications are found of a decreased membrane permeability in anoxic brain slices. The observation that an increased potassium efflux is evoked by potassium concentrations which are too low to affect energy metabolism and swelling markedly, and that the effect may be observed in neonatal tissue are not per se in discordance with the hypothesis of a potassium-induced active accumulation of potassium (cf. however also Schousboe & Hertz, 1971b).

Part of the potassium movements seems to be accompanied by sodium movements in the opposite direction as shown by the augmentation of the sodium concentration after application of electrical pulses or addition of more than 20 mM potassium, and by the concomitant decrease in sodium concentration and sharp rise in potassium concentration at about 20 mM external potassium. The reaccumulation of potassium and the re-extrusion of sodium after termination of electrical stimulation seem also to suggest some exchange between sodium and potas-

sium ions, but no electrically induced or potassium-induced increase was found in the rate constant of the sodium efflux, whereas the potassium influx was at least doubled or tripled compared to the values in a "normal", "balanced" medium. It should, however, be kept in mind that the sodium concentration in the tissue becomes somewhat increased (about 50%) after electrical stimulation or exposure to excess potassium (probably as a result of an increased influx), and that also the efflux (which equals rate constant multiplied by the concentration) thus is somewhat raised. This will per se tend towards a reestablishment of the previous sodium level after the cessation of the increased sodium influx. Nevertheless the effect by potassium on potassium fluxes seems to be quantitatively much more important than its influence on sodium fluxes. This dissociation between potassium and sodium transport seems of interest, since the two ions in several cases are supposed to be exchanged against each other. Indications are, however, found in the literature that sodium and potassium transport may not always be too closely interconnected in brain (e. g. Hertz, 1968; cf. also Swanson, 1968; Harvey & McIlwain, 1968).

A redistribution of potassium ions via glial cells was first suggested on the basis of morphological and electrophysiological evidence (Horstmann & Meves, 1959; Ranck, 1964) and of the cellular distribution of the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ (Cummins & Hydén, 1962) and is in keeping with the great potassium content in the presumably neuroglial compartment observed after incubation in potassium-rich media (Franck, 1970; IV B, 5a). The role of the glia cells for the maintenance of potassium homeostasis in the extracellular fluid has been discussed by several investigators (e. g. Hertz, 1965a, b, 1966, 1968, 1973; Orkand et al., 1966; Kuffler & Nicholls, 1966; Kuffler, 1967; Bourke, 1969b; Trachtenberg & Pollen, 1970; Lund-Andersen & Hertz, 1970; Orkand, 1970; Okamoto & Quastel, 1970b; Schousboe & Hertz, 1971b; Haljamäe & Hamberger, 1971; Henn et al., 1972; Trachtenberg et al., 1972; cf. however also Wendell-Smith & Blunt, 1965), and three more or less related concepts have emerged:

- 1) A passive redistribution through glia cells of potassium ions carried by the electrical currents (Kuffler and coworkers, 1966-1970; Trachtenberg & Pollen, 1970),
- 2) an accumulation of potassium ions into glia cells mediated by the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ (Hamberger and coworkers, 1971-1972), and
- 3) a coupled accumulation of chloride and potassium ions into glia cells, which for osmotical reasons also leads to an uptake of water (Hertz and coworkers, 1965-1973; Bourke and coworkers, 1969-1972).

The hypothesis of a passive redistribution is experimentally well founded, since it has been directly observed that currents flow through the glial membrane

as a result of neuronal activity, and that the membrane is permeable to potassium ions (IV B, 4a), but its quantitative significance cannot be evaluated; this hypothesis is founded upon experiments with glial cells from invertebrates or from white matter, and the universal applicability of the observations has not been ascertained. The hypothesis does not explain the metabolic effects of excess potassium, the specific, neuroglial localization of the potassium-induced swelling or the high potassium concentration in glia cells in vitro (IV A, 3c). The two other hypotheses both involve mediated, probably active transport, and are thus compatible with the potassium effects on energy metabolism. In rat brain-cortex slices, the potassium-induced or electrically induced increase in the rate of oxygen consumption amounts to about 50 μ moles/g initial wet wt./hr (Fig. II, 5) which corresponds to approximately 30-40 μ moles/g final wet wt./hr or 0.5 μ moles/min, and the corresponding increase in potassium fluxes is about 5-10 μ moles/g/min. The ratio between moles of potassium transported, and moles of oxygen consumed (K/O_2 ratio) becomes accordingly 10-20, which is close to the Na/O_2 ratio of 18 observed in frog skin (Zerahn, 1956; cf. also Leaf & Renshaw, 1957). It has often been suggested that the potassium-induced stimulation of oxygen uptake should be a result of the depolarization (e.g. Marchbanks, 1970). The resistance of the metabolic response to deviations in incubation conditions (indicated by the agreement between different investigators with respect to the metabolic stimulation) contrasts, however, the sensitivity of the polarization to environmental factors (cf. IV A, 3d) and is thus against this concept. The low concentration of cesium required to increase the oxygen uptake (II B, 2a) and the swelling (IV B, 2b) does probably also not depolarize the cells. An ion-induced activation of an enzyme may both be more likely (cf. II B, 2a; II C and VI B, 4c) and biochemically meaningful, and the correlation between the neuroglial localization of the Na^+-K^+ -ATPase and the ability of glia cells to accumulate potassium is obvious (Henn *et al.*, 1972). The potassium concentrations (about 10-20 mM) required for a maximum stimulation of the ATPase (VI B, 4c) are, however, smaller than those (> 20 mM) affecting energy metabolism (II B, 2a, III B, 2a, VI B, 3b; cf. also Hertz, 1973). A dissociation between potassium effects on potassium and sodium transport should also not be expected during stimulation of this enzyme, and the hypothesis does not explain the selective glial localization of the potassium-induced swelling. The correspondance between the concentrations of potassium (20-50 mM) which cause a rise in the rate of oxygen uptake (cf. Fig. II, 5), a decrease in the concentration of ATP (III B, 2a) and an increase in the degree of the swelling (Fig. IV, 5), as well as the indispensability of sodium for all three phenomena indicate a close correlation between swelling and stimulation of energy metabolism and is in favour of a coupled uptake of potassium, chloride and water into neuroglia. According to Bourke (1969a, b) the ion

species directly affected is the chloride ion, whereas Hertz (1965a, 1966, 1968; Lund-Andersen & Hertz, 1970) envisages a stimulation of the transport of potassium ions. In either case the two ions must travel together, and the discrepancy has no neurobiological implications. An active transport of potassium ions is not compatible with the concept that the glia cells involved behave as perfect potassium electrodes, whereas an active chloride uptake would be meaningless into cells with a high permeability to chloride. It is therefore of importance that it at least can be stated with reasonable certainty that both ions are not in electrochemical equilibrium in brain tissue (IV A, 3d; cf. also Allen, 1955).

The three hypotheses discussed above, all serve the purpose of clearing the neuronal surroundings from excess potassium, and none of them gives an immediate answer to the further fate of the displaced potassium ions. The extent of the passive, current-carried transport demonstrated by Kuffler and coworkers must be anatomically determined by the presence of intercellular junctions, whereas the enzyme-carried transport mechanisms rapidly may be altered by enzyme induction (cf. Hertz, 1965a). The latter may conceivably represent a further, more flexible and efficient ontogenetic development from the system found in the leech (cf. also Trachtenberg & Pollen, 1970), and the possible physiological implication of a potassium transport through glial cells has been discussed (Hertz, 1965a, b; Adey, 1969, p. 112). Ultimately the released potassium ions must be re-accumulated into the neurons in order to maintain their potassium content and polarization. None of the three hypotheses deals with the mechanism involved in this transport.

A correlation was indicated between the potassium-induced stimulation of oxygen uptake and spreading depression (cf. also Hertz, 1965a), and both potassium ions (IV A, 2f) and glutamate ions (Van Harreveld & Fífková, 1970 ; V B, 1c) have been suggested as mediators of the latter phenomenon. A discrepancy seems, however, to exist between the neuroglial swelling evoked by excess potassium or glutamate (IV B, 2b and c) and the dendritical swelling and astrocytic shrinking during spreading depression. The methodological difficulties in determination of the volume of the glial processes (IV A, 2f) should, however, be kept in mind, and asphyxia which in several respects mimics the changes brought about by spreading depression (cf. e. g., Van Harreveld, 1966 p. 121) has been observed to cause a swelling of the, probably neuroglial, Bergmann fibers in cerebellum (Van Harreveld, 1961). If spreading depression is accompanied by an increased potassium concentration in an isosmotic extracellular fluid, all depolarized structures can be expected to swell (cf. Boyle & Conway, 1941).

Nitrogen Metabolism

A. AMINO ACIDS, TRANSMITTERS, PROTEIN AND NUCLEIC ACIDS IN BRAIN

1. Interrelations between ions and nitrogeneous compounds

Certain amino acids (e.g. glutamate) exert effects which resemble those evoked by deviations in the ionic composition of the media (e.g. Rossiter, 1955 - cf. also II B, 2a; III B, 6; IV B, 2c; IV B, 3a-b; IV B, 4c; VI B, 3b). The concentrations of some ions (predominantly sodium and potassium) are, on the other hand, of major importance for the turnover of the amino acids in several tissues including brain, (e.g. Christensen, 1970), and for the formation of protein and RNA (cf. below). The transmitters norepinephrine, serotonin and acetylcholine are amino acid derivatives. The latter probably owes at least part of its action to effects on membrane permeability for ions (e.g. Hebb & Krnjević, 1962), and the transmitter release is affected by the ion composition of the medium.

2. Amino acids and their derivatives in brain

a. In vivo: Several amino acids are found in the brain in high concentrations. Acetyl aspartic acid and γ -amino-butyric acid (GABA) which do not occur in significant concentrations in other tissues in the mammalian body, thus reach concentrations between 1 and 10 μ moles/g wet wt. (e.g. Waelsch, 1962; McIlwain, 1966, p. 128). The glutamine concentration is 4-5 μ moles/g and the concentration of glutamic acid is higher (about 10 μ moles/g) than in any other organ (e.g. Tower, 1960; Mangan & Whittaker, 1966; Van den Berg, 1970b). The latter amino acid is rapidly formed from glucose in rats older than 2-3 weeks (Gaitonde & Richter,

1966 - cf. also Van den Berg, 1970a and Fig. V, 2) and is formed in at least two, only partly intercommunicating compartments (Fig. V, 1), i.e. a larger pool comprising most of the glutamate (and associated tricarboxylic acid cycle) and a smaller pool (with associated tricarboxylic acid cycle) from which glutamine is formed (e.g. Lajtha, Berl & Waelsch, 1959; Berl *et al.*, 1961; Garfinkel, 1962, 1966; Gaitonde, 1965; O'Neal & Koeppe, 1966; Van den Berg, Krzalic, Mela & Waelsch, 1969; Berl & Clarke, 1969; Rose, 1970; Van den Berg & Garfinkel, 1971). Evidence is found (Van den Berg & Garfinkel, 1971) that the two pools may

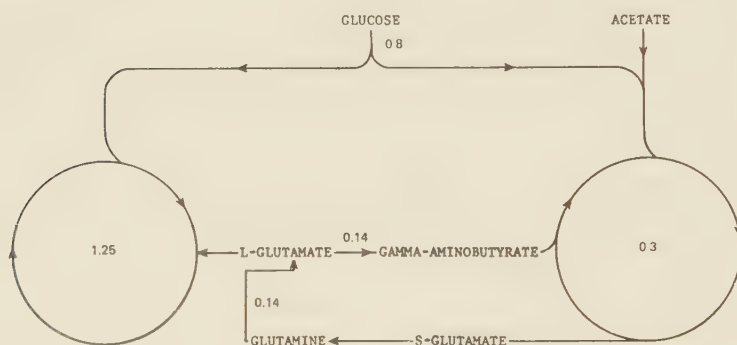


Fig. V, 1. Overall scheme of compartmentation model for the mouse brain *in vivo*. Values are fluxes in $\mu\text{moles/g wet wt. per min.}$ (cf. VI A, 2) and L- and S-glutamate designate the glutamate of the large, respectively the small compartment. From Van den Berg & Garfinkel, 1971.

be partly connected by a flow of glutamine from the small to the large compartment (Fig. V, 1) and a movement of GABA in the opposite direction (cf. also Berl, Nicklas & Clarke, 1970; Benjamin & Quastel, 1972). The metabolic compartmentation is absent (Fig. V, 2) in new-born kittens or rats (Berl, 1965; Patel & Balazs, 1969, 1970), where the concentrations of glutamate, aspartate and GABA (but not of glutamine) are relatively low (e.g. Berl & Purpura, 1963; Berl, 1965; Agrawal, Davis & Himwich, 1966; Patel & Balazs, 1970), and it has been suggested that this may be due to a reduced operation of the large compartment neonatally (Patel & Balazs, 1970; Van den Berg, 1970a).

Conceivably the compartmentation could be due to metabolic differences be-

tween, e.g. mitochondria and cytoplasm, cell body, axon and synaptosome or neurons and glia cells (e.g. Berl & Clarke, 1969; Rose, 1970; Patel & Balazs, 1970; Balazs, 1971 - cf. also I B, 5). It has been suggested that a considerable fraction of the glutamine content should be present in non-neuronal elements (Tower, 1960; Margolis, Heller & Moore, 1968; Benjamin & Quastel, 1972; cf. also Bradford & Thomas, 1969), whereas ontogenetic and topographic findings indicate that GABA mainly is formed in neurons (Schadé & Baxter, 1960; Baxter, Schadé & Roberts, 1960; Tower, 1960; McKhann, Albers, Sokoloff, Mickelsen & Tower, 1960; Berl & Purpura, 1963; Balazs, Patel, Johnson & Richter, 1971; Roberts, 1971). A characteristic L-glutamic acid decarboxylase is, however, also present in glia cells (Haber, Kuriyama & Roberts, 1970), and studies on the degenerated medial geniculate body have indicated that GABA may be more or less equally distributed between neurons and glia cells (Utley, 1963; Margolis *et al.*, 1968; cf. however also Wollemann & Devenyi, 1963). Evidence is also found that GABA and glutamic acid may not be preferentially localized in nerve

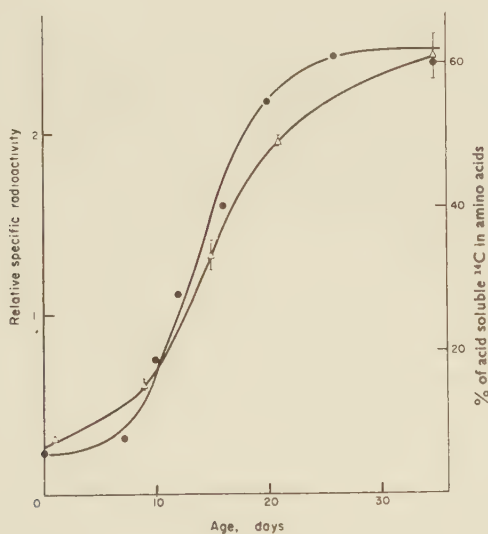


Fig. V, 2. In vivo changes in the specific radioactivity of glutamine relative to that of glutamate (RSA)Δ, and in conversion of ¹⁴C from glucose into amino acids (% of acid soluble ¹⁴C in amino acids after 20 min.), ●, during the ontogenetic development of rats. Standard errors are shown for Δ. Compartmentation is indicated by a RSA > 1. From Patel and Balazs (1970).

terminals (Ryall, 1964; Mangan & Whittaker, 1966 - cf. however also Salganicoff & De Robertis, 1965; Balazs, Dahl & Harwood, 1966; Neal & Iversen, 1969),

though these compounds have several characteristics in common with ordinary transmitters (e.g. Krnjević, 1965; Galindo, Krnjević & Schwartz, 1967). The synaptosomally located amount of glutamate and GABA, is, on the other hand, probably sufficient to exert a transmitter function (Krnjević & Whittaker, 1965; Mangan & Whittaker, 1966).

The acetylcholine content in brain is only about 1/10 to 1/100 of that found in sympathetic ganglia (McIlwain, 1966, p. 300), but the transmitter probably plays a physiological role in the central nervous system, and addition of acetylcholine depolarizes at least some neurons and glia cells (Krnjević & Schwartz, 1967; cf. however also Wardell, 1966). The acetylcholine is present in two different forms, i.e. bound (pharmacologically inactive), and free (pharmacologically active) acetylcholine. Some of the ionic effects to be described below (e.g. that of potassium) may partly consist in a conversion of bound acetylcholine to free acetylcholine. Both the concentration of acetylcholine, the choline esterase activity and the amount of acetylcholine released per g brain tissue decrease with an increasing brain weight (Tower & Elliott, 1952; Tower & Young, 1973) i.e. show the same species dependence as the neuronal density (Fig. V, 3 - cf. also Tower, 1954 and I B, 4).

Spreading depression can be evoked by topical application to the exposed cortex of 15 mM L-glutamate or 4 mM D-glutamate (Van Harreveld, 1959). Also certain other amino acids are effective, and a number of amino acids are released from the exposed rabbit brain as a result of the depression (Van Harreveld & Kooiman, 1965). Concomitantly, the incorporation of labelled amino acid into protein is decreased (Bennett & Edelman, 1969).

The role of proteins and nucleic acid in brain function has received increasing attention in recent years (e.g. Hydén, 1967b, c; Lodin & Rose, 1968; Altman, 1969; Richter, 1970). Certain proteins seem to be localized mainly or exclusively in either neurons or neuroglial cells. S-100 has thus a predominant neuroglial localization (Hydén & McEwen, 1966) and is not found in rats until the age of about two weeks (Moore, 1969; Herschman, Levine & De Vellis, 1971). By far the major part of the overall protein synthesis in vivo seems, however, to occur in the neurons (e.g. Blomstrand & Hamberger, 1969; Blomstrand, 1971).

b. In vitro: An uptake against a concentration gradient has been demonstrated with e.g. D- and L-glutamic acid (Stern, Eggleston, Hems & Krebs, 1949; Takagaki et al., 1959; Tsukada et al., 1963; Margolis & Lajtha, 1968; Okamoto & Quastel, 1972), GABA (Elliott & Van Gelder, 1958; Tsukada et al., 1963; Iversen & Neal, 1968; Balázs et al., 1970), glutamine (Stern et al., 1949; Tsukada et al., 1963), D- and L-aspartic acid (Tsukada et al., 1963; Margolis & Lajtha, 1968); β -alanine (Tsukada et al., 1963), glycine (Abadom & Scholefield, 1962a), tyrosine

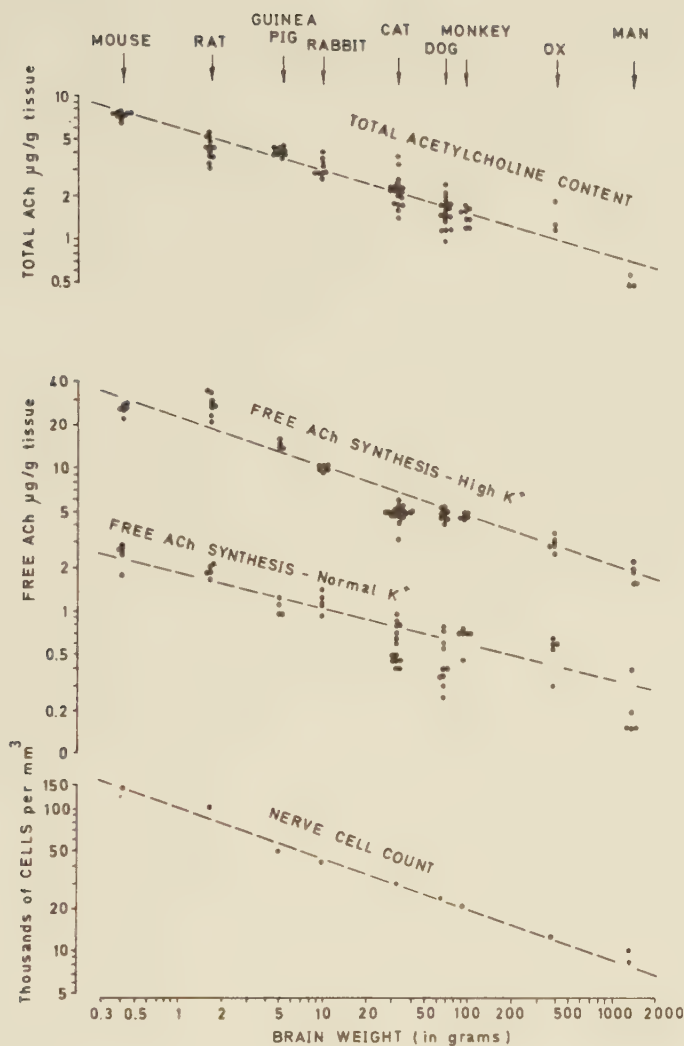


Fig. V, 3. Correlations between brain weight in different species and total acetylcholine content in brain cortex samples (measured 5 min after the excision), acetylcholine release (Free Ach synthesis) from brain-cortex slices during incubation (1 hr) in "balanced" and in potassium-rich media, and nerve cell counts (from the motor cortex).

Both axes are drawn on logarithmic scales. After Tower & Elliott (1952).

Guroff, King & Udenfriend, 1961), L-histidine (Neame, 1961; De Almeida, Chain & Pocchiari, 1965); L-leucine (Margolis & Lajtha, 1968); L-lysine (Neame, 1961; Margolis & Lajtha, 1968); proline, arginine, ornithine, methionine (Neame, 1961), valine (Jones & Banks, 1970b) and 5-hydroxytryptophan (Schanberg & Giarman, 1960; Smith, 1963). The uptake is dependent upon the presence of oxygen and a suitable substrate. The external concentration of the amino acid is of importance for the uptake and thus for the concentration obtained in the tissue (Stern *et al.*, 1949). Considerable differences (Fig. V, 4) are found between the individual amino acids (Margolis & Lajtha, 1968), and the length of the incubation period (Fig. V, 5 and 6) is of importance for the concentration obtained (Stern *et al.*, 1949; Tsukada *et al.*, 1963). The correlation between ATP level and the uptake of amino acids has been studied by several investigators, e.g. Abadom & Scholefield (1962a), Piccoli, Grynbaum & Lajtha, (1971), and Banay-Schwartz *et al.* (1971).

The glial-enriched fraction obtained by gradient centrifugation shows a smaller pool size but a greater uptake of most amino acids than the corresponding neuronal-enriched fraction (Bradford & Rose, 1967; Rose, 1968). Among the amino acids which are accumulated to a considerable degree into glia cells is GABA, which may indicate an inactivation of transmitters by uptake into glia cells (Henn & Hamberger, 1971 - cf. also Koelle & Koelle, 1959; Koelle, 1962; Krnjević & Schwartz, 1967; Hökfelt & Ljungdahl, 1971; Okamoto & Quastel, 1972).

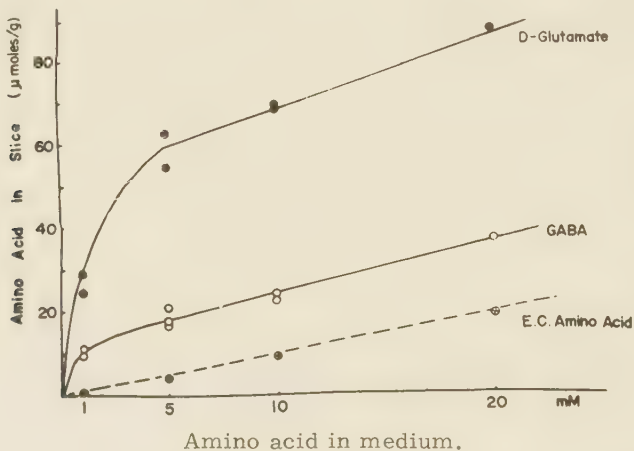


Fig. V, 4. Accumulation of D-glutamate and of GABA into guinea pig brain-cortex slices as a function of the concentration of the amino acid in a "balanced" medium. The lower, broken line indicates the probable content of the amino acid in the extracellular space of the slice which was calculated from the chloride space (cf. IV A, 3a). From Tsukada *et al.* (1963).

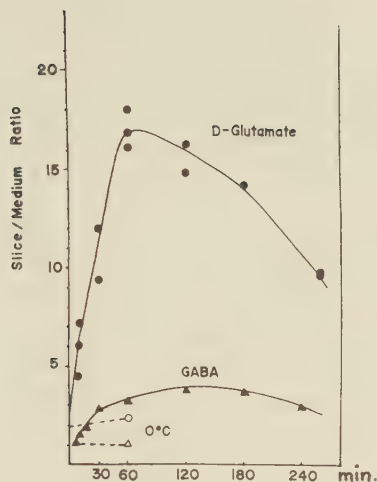


Fig. V, 5. Accumulation (slice/medium ratio) of D-glutamate and of GABA in guinea pig brain-cortex slices as a function of the time of incubation at 37° and at 0° in a "balanced" medium containing 5 mM of the amino acid in question.

From Tsukada et al. (1963).

Incorporation of radioactivity from ^{14}C -labelled glucose into especially glutamate (cf. Vrba, Gaitonde & Richter, 1962), but also into aspartate, GABA, glutamine, serine and alanine (Belloff-Chain, Catanzaro, Chain, Masi & Pocchiari, 1955; Kini & Quastel, 1959; Balázs et al., 1970) occurs rapidly. The incorporation is faster in the glial-enriched than in the neuronal-enriched fraction (Rose, 1968); only little glutamine is formed in synaptosomes (Bradford & Thomas, 1969). Metabolic compartmentation (cf. V A, 2a) has been observed in slices after addition of ^{14}C labelled acetate, bicarbonate, aspartate, glutamate or GABA (e.g. Machiyama, Balázs & Richter, 1967; Berl et al., 1968; Berl & Clarke, 1969; Balázs et al., 1970; Lund-Andersen, Arnfred & Hertz, 1971) to a medium containing glucose, but disappears after homogenization (Berl & Clarke, 1969). The amino acids of the "small" compartment labelled with GABA seem to show a rapid exchange with the medium (Balázs et al., 1970). Formation of glutamine from glutamate has been observed both in brain extracts and in brain slices (W. H. Elliott, 1951; Acs et al., 1953) and mainly the glutamate of the "small" compartment is involved (Balázs et al., 1970). During incubation of slices the content of glutamine may increase both in the tissue (Folbergrová, 1963) and in the medium (Balázs et al., 1970). The maximum, short-lasting, rate of glutamine synthesis is 50 $\mu\text{moles/g wet wt. /hr.}$ (Woodman & McIlwain, 1961) and the formation of glutamine may be of importance in decreasing the concentration of the toxic am-

monium ion (cf. Du Ruisseau, Greenstein, Winitz & Birnbaum, 1957; Berl, Takagaki, Clarke & Waelsch, 1962), which seems to become formed as a result of stimulation (Vladimirova, 1938; Richter & Dawson, 1948; Tsukada, Takagaki, Sugimoto & Hirano, 1958). The other derivative of glutamate (and possible link between the compartments), GABA is an intermediate in the GABA-shunt, through which ketoglutarate in the brain may be converted to succinate (Roberts, 1956; cf. VI A, 2).

Also incorporation of labelled glucose or amino acids into proteins occurs in vitro (e.g. Winzler, Moldave, Rafelson & Pearson, 1952; Roberts, Flexner & Flexner, 1959; Waelsch & Lajtha, 1961; Orrego & Lipmann, 1967; Jones & Banks, 1970a). The pH maximum is around 7.1 (Mase, Takahashi & Ogata, 1962), and the most active synthesis takes place in the neurons (e.g. Blomstrand & Hamberger, 1970; Blomstrand, 1971). Similarly, labelled uridine may be incorporated into RNA in brain slices (Prives & Quastel, 1967; Huttunen, 1969) and the synthesis is especially fast in tissue from young animals (Orrego, 1967).

B. ION EFFECTS ON NITROGENEOUS COMPOUNDS

1. Exchange between tissue and medium

a. Measurement of ion effects on amino acid fluxes and metabolism: Exact measurements of rates of uptake or release of amino acids (or other substances) may be obtained by following the influx or the efflux of the labelled compound with time (cf. III B, 2b and IV A, 3e), and the effects of e.g. excess potassium or electrical stimulation on amino acid fluxes have been extensively studied in this way (V B, 1c-d). In many investigations the accumulation of amino acids has, however, been estimated by aid of slice/medium ratios or simply by the concentrations obtained in the tissue (cf. Figs. V, 4 and V, 5). This procedure has the disadvantage that no conclusions can be made, whether changes evoked by, e.g. modification of the ionic composition of the medium are brought about by alterations in influx or in efflux. Furthermore, the concentrations - and the specific activities - are changed not only by factors which affect the in- or efflux, but also by factors which change the rate of the metabolic turnover of the substance (Margolis & Lajtha, 1968; cf. also V B, 2). Under suitable conditions such a distinction may, however, be made by concomitant determination of the amino acids of the medium (cf. Machiyama et al., 1970).

b. Sodium effects: The presence of the sodium ion has been found necessary for the accumulation of D-glutamate (Takagaki et al., 1959), L-glutamate (Margolis & Lajtha, 1968), D- and L-aspartate (Margolis & Lajtha, 1968), GABA (Iversen & Neal, 1968), glycine (Abadom & Scholefield, 1962b; Nakazawa & Quastel, 1968a), D- and L-leucine, D- and L-lysine (Lahiri & Lajtha, 1964; Margolis & Lajtha, 1968), α -aminoisobutyric acid and diaminobutyric acid (Margolis & Lajtha, 1968). In the case of valine it has been shown that sodium deficiency both decreases the influx and increases the efflux (Jones & Banks, 1970b). Other ions can only poorly substitute for sodium (Abadom & Scholefield, 1962b; Lahiri & Lajtha, 1964; De Almeida et al., 1965), but differences may exist between the individual amino acids. Also the stimulation of valine influx after application of electrical pulses (V B, 1d) is dependent upon the sodium concentration (Jones & Banks, 1970b). The production of acetylcholine (measured as the sum of the free and bound transmitter) is seriously interfered with in sodium-free media, but its release from stimulated ganglia is even more decreased than can be explained as a result of the impaired synthesis (Birks, 1963). It was accordingly suggested by Birks (1963) that the increase in the intracellular sodium concentration during the stimulation may trigger the transmitter release. The reduction of the acetylcholine release in sodium deficient media may be delayed by some minutes which explains the observation by Hutter and Kostial (1955), that the acetylcholine release was not affected in their relatively short-lasting experiments.

A low sodium concentration during the incubation of brain-cortex slices causes a reduction of the incorporation of leucine into proteins (Blomstrand & Hamberger, 1970), which conceivably could be related to the impairment of leucine accumulation. Incubation of cell-enriched fractions indicate a greater susceptibility to sodium lack in glial cells than in neurons (Blomstrand, 1971). The incorporation of ^{14}C labelled uridine into RNA and nucleotides is reduced both in sodium-deficient media and in media containing excess sodium (Prives & Quastel, 1969).

c. Potassium effects: The absence of potassium decreases the slice/medium ratio of L-glycine (Abadom & Scholefield, 1962b), D- and L-glutamate (Takagaki et al., 1959; Tsukada et al., 1963; Margolis & Lajtha, 1968), D-aspartate (Takagaki, 1963), L-aspartate (Margolis & Lajtha, 1968), L-glutamine and GABA (Tsukada et al., 1963), and leads to an increased output of acetylcholine from the perfused ganglion (Birks, 1963). The accumulation of D-glutamine and of lysine, leucine and aminoisobutyrate has been reported to be unaffected during incubation in media prepared without potassium (Tsukada et al., 1963; Lahiri & Lajtha, 1964), but it must be recalled that small (1-2 mM) concentrations of potassium may rapidly be obtained in "potassium-free" media by leakage from the tissue (cf. II B, 1a), and Margolis & Lajtha (1968) observed a pronounced inhibition of

the uptake of all amino acids studied (including leucine, lysine and isobutyric acid derivatives) in experiments where the media were kept potassium-free by medium exchange. This inhibition was unrelated to the concomitant decrease in the concentration of ATP (cf. also Jones & Banks, 1970b).

High concentrations of potassium (i. e., about 30-100 mM) have apparently no effect on leucine uptake (Lahiri & Lajtha, 1964) but do cause a lowered accumulation of several amino acids including tyrosine (Guroff, King & Udenfriend, 1961), glycine (Abadom & Scholefield, 1962b; Nakazawa & Quastel, 1968b), histidine (De Almeida *et al.*, 1965), lysine, aminoisobutyrate, and cycloleucine (Lahiri & Lajtha, 1964). Also the slice/medium ratio of D- and L-glutamate (Fig. V, 6)

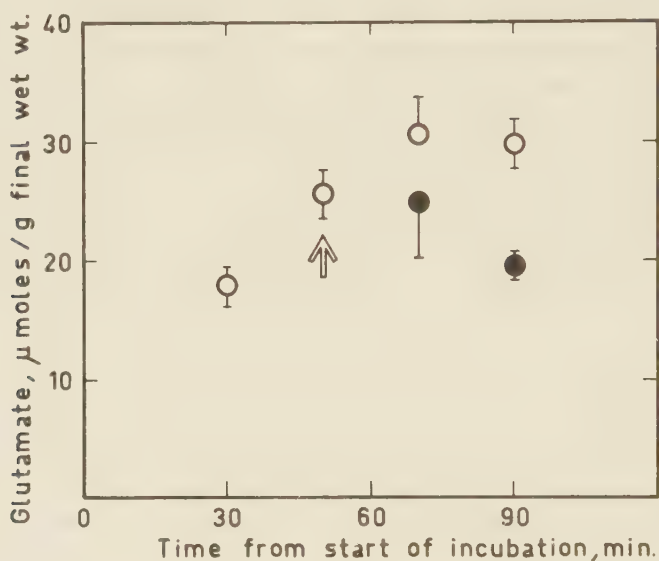


Fig. V, 6. Concentration of L-glutamate in rat brain-cortex slices as a function of the time of incubation in a medium containing 10 mM L-glutamate. All slices were initially incubated in a medium containing 5 mM potassium (o); half of the slices (o-o) remained in this medium, whereas potassium chloride to a final concentration of 55 mM was added to the other half (●) at arrow. Results are means of 4-8 experiments with S.E.M. indicated by vertical bars. After Arnfred & Hertz, 1971

and of GABA is decreased in potassium-rich media (Takagaki *et al.*, 1959; Tsukada *et al.*, 1963; Arnfred & Hertz, 1971; cf. however also Benjamin & Quastel, 1972), whereas that of glutamine is considerably increased (Tsukada *et al.*, 1963; Machiyama *et al.*, 1970; Hamajima, 1970).

The decreased content of glutamate after incubation in glucose-containing potassium-rich media is correlated with an increased efflux (Fig. V, 7 - cf. also IV A, 3e) of this amino acid from preparations of brain-cortex or retina loaded with ^{14}C -glutamate (Hertz & Arnfred, 1967; Hertz, 1968, 1969; Katz, Chase & Kopin, 1969; Bradford, 1970; Van Harreveld & Fifková, 1970; Arnfred & Hertz, 1971). A similar potassium induced release of endogeneously formed glutamate has been observed in synaptosomes (De Belleruche & Bradford, 1972) but is much smaller in brain slices (Machiyama et al., 1967; Machiyama et al., 1970). By aid of chromatography it has been shown that the increased amount of ^{14}C , which is released from labelled glutamate, largely is due to an efflux of unmetabolized glutamate (Van Harreveld & Fifková, 1970; Arnfred & Hertz, 1971), and glutamate was suggested as the chemical mediator of spreading depression (Van Harreveld & Fifková, 1970). The potassium-induced release of radioactive glutamate is enhanced under anoxic conditions or by the presence of high concentrations of glutamate in the medium, which conceivably could be explained by a re-uptake of glutamate in the tissue (cf. De Belleruche & Bradford, 1972). The effect is absent in slices from new born rats (Arnfred & Hertz, 1971). The potassium ion can not be replaced by sodium (Arnfred & Hertz, 1971), and lithium in a low concentration inhibits the response (Katz et al., 1969). Also the influx of glutamate appears to be slightly increased by excess potassium, but the effect is quantitatively less pronounced (Arnfred & Hertz, 1971; cf. also Quastel, 1959). After labelling with ^{14}C glutamine the release of radioactivity is unaffected by excess potassium (Hertz, 1969; Arnfred & Hertz, 1971), but glutamine formed from radioactive GABA is rapidly equilibrated with the medium in potassium-rich media (Machiyama et al., 1970).

The efflux of GABA is increased by excess potassium (Machiyama et al., 1967; Srinivasan, Neal & Mitchell, 1969; Machiyama et al., 1970; Arnfred & Hertz, 1971). The effect is transitory, unless glutamate is also present (Fig. V, 7). Ammonium or sodium is not able to replace potassium (Machiyama et al., 1970), and small concentrations of lithium cause a considerable reduction of the stimulated GABA efflux (Katz et al., 1969). In contrast to glutamate and glutamine, GABA shows a reduced uptake in potassium-rich media (Machiyama et al., 1970). Also after labelling of brain-cortex slices with ^{14}C -containing aspartate, excess potassium increases the release of radioactivity (Hertz, 1969), but high potassium concentrations have in the absence of glutamate (cf. V B, 1e) no effect on the tracer efflux after "loading" with labelled leucine and lysine (Arnfred & Hertz, 1971).

Addition of excess potassium causes a liberation of acetylcholine from the perfused ganglion (Feldberg & Vartiainen, 1934; Brown & Feldberg, 1936; McLennan & Elliott, 1950; Hutter & Kostial, 1955; Birks, 1963), from the isolated ganglion

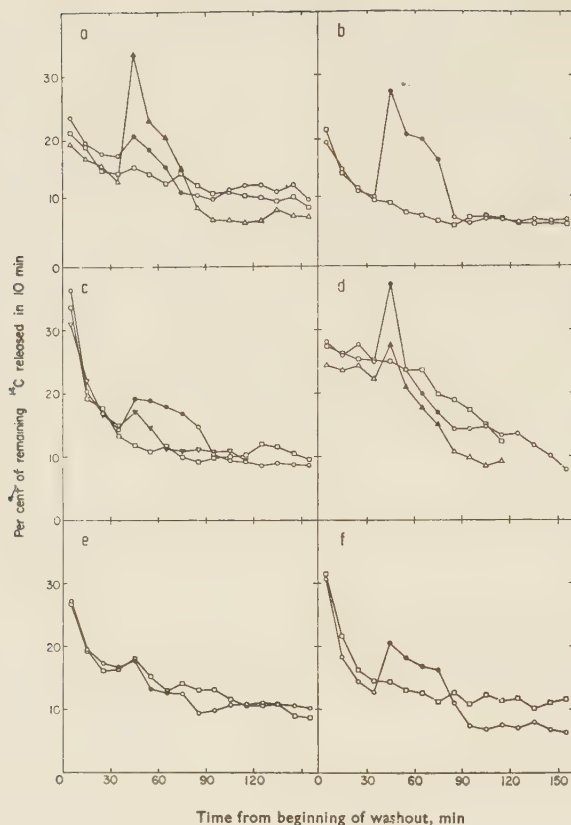


Fig. V, 7. Release of ^{14}C per 10 min period during wash-out into a non-radioactive medium. Rat brain-cortex slices were "loaded" with labelled amino acid during a 30 min preincubation period in an oxygenated medium containing 6 mM glucose, and the ^{14}C subsequently washed out by incubation for 10 min periods in a series of test tubes bubbled with $\text{O}_2 + \text{CO}_2$ (aerobic) or $\text{N}_2 + \text{CO}_2$ (anaerobic). When the effect of additions to the medium was tested, the four test tubes in which the slices were incubated between 40 and 80 min, contained medium with added KCl, NaCl or glutamate. The curves are averages of two to seven individual, essentially similar experiments. Number of experiments in brackets.

a. ^{14}C -glutamate, "balanced" glucose-containing medium, ($\square, \circ, \triangle$) with 50 mM KCl ($\bullet$) or 10 mM glutamate ($\blacktriangle$) added (4, 4, 4).

b. ^{14}C -glutamate, "balanced" glucose-containing anaerobic medium ($\square, \circ$) with 50 mM KCl added ($\bullet$) (3, 4).

c. ^{14}C -glutamate, medium with 6 mM glucose, 10 mM glutamate ($\square, \circ, \nabla$) plus 50 mM KCl ($\bullet$) or 50 mM NaCl ($\blacktriangledown$) (2, 5, 5).

Fig. V, 7. (Continued)

d. ^{14}C -GABA, "balanced", glucose-containing medium ($\square$, $\circ$, Δ), 50 mM KCl ($\bullet$) or 10 mM glutamate ($\blacktriangle$) added (7, 3, 4).

e. ^{14}C -glutamine, "balanced", glucose-containing medium ($\square$, $\circ$) with 50 mM KCl added ($\bullet$) (3, 3).

f. ^{14}C -glutamine, medium with 6 mM glucose, 10 mM glutamate ($\square$, $\circ$) and 50 mM KCl added ($\bullet$) (3, 3).

From Arnfred & Hertz, 1971.

(Lipicky *et al.*, 1963) and from brain slices (Mann, Tennenbaum & Quastel, 1939; Welsh & Hyde, 1944), but not from brain homogenates (Mann *et al.*, 1939; McLennan & Elliott, 1952; Elliott, 1955; Sharkawi & Schulman, 1969). The effect is present in slices from new-born rats (Welsh & Hyde, 1944). Rubidium and cesium resemble potassium in stimulating the acetylcholine liberation from the perfused superior cervical ganglion of the cat (Brown & Feldberg, 1937) and from brain slices (Mann *et al.*, 1939). Quantitatively, rubidium and potassium exert almost equal effects, whereas the cesium ion seems to be less active. The presence of CO_2 /bicarbonate is essential for a maximum response (Birks & McIntosh, 1961; Mann *et al.*, 1939; McLennan & Elliott, 1950), and no potassium induced acetylcholine release is observed under anoxic conditions (Welsh & Hyde, 1944). Also the release of norepinephrine from brain slices is increased by excess potassium (Baldessarini & Kopin, 1966, 1967).

A minor increase of the potassium concentration, i. e. to 15-40 mM, may lead to an increased incorporation of radioactive precursors into RNA (Prives & Quastel, 1967, 1969; Huttunen, 1969) and protein (Blomstrand & Hamberger, 1970) in brain-cortex slices, but greater concentrations lead to a reduction (Bassi & Bernelli-Zassera, 1960; Mase *et al.*, 1962; Prives & Quastel, 1967, 1969; Huttunen, 1969; Blomstrand & Hamberger, 1970), and topical application of potassium chloride to the exposed rat cortex leads to a decrease in the content of RNA (Ruscák & Ruscáková, 1968). Incubation of separated cell fractions have failed to show any stimulation of leucine incorporation into proteins (Blomstrand, 1971); the incorporation into the neuronal fraction is unaffected by excess potassium and that into neuroglia decreased by excess potassium (Takahashi, Hsü & Honma, 1970).

d. Electrical stimulation: Application of electrical pulses resembles excess potassium in raising the efflux of glutamate, aspartate and GABA from brain slices, synaptosomes or retina (Katz *et al.*, 1969; Srinivasan *et al.*, 1969; Bradford, 1970; Van Harreveld & Fifková, 1970; De Belleruche & Bradford, 1972).

The effect is specific for nervous tissue (Katz *et al.*, 1969), and a small loss of glutamate and aspartate from peripheral nerve is not affected by an increase in the potassium concentration or by electrical stimulation (Lewis, 1954). Both influx and efflux of L-valine may show a transitory increase during electrical stimulation and the valine concentration becomes decreased (Jones & Banks, 1970b). The level of glycine has, in contrast, been reported to be unaffected by electrical stimulation of brain slices (Nakazawa & Quastel, 1968a). The release of acetylcholine (Rowell, 1954), norepinephrine (Baldessarini & Kopin, 1966), serotonin (Chase, Breeze & Kopin, 1967) and adenine derivatives (Pull & McIlwain, 1972) from brain slices is increased by application of electrical pulses.

Electrical stimulation may evoke an initial, transient increase (Jones & Banks, 1970a), and a subsequent reduction of amino acid incorporation into protein (Orrego & Lipmann, 1967; Huttunen, 1969) and a considerable decrease in the formation of RNA from uridine (Orrego, 1967; Prives & Quastel, 1969) or orotic acid (Huttunen, 1969).

e. Glutamate effects: The addition of 10 mM L-glutamate (Fig. V, 7) leads to an increase in the rate constant of the glutamate efflux from brain-cortex slices or retinae previously loaded with ^{14}C glutamate (Arnfred & Hertz, 1971; Van Harreveld & Fiková, 1971a). The presence of glutamate in the medium gives also rise to a potassium-induced stimulation of the efflux of the amino acids glutamine, leucine and lysine, on which excess potassium has no effect in the absence of glutamate (Srinivasan *et al.*, 1969; Arnfred & Hertz, 1971). The presence of glutamate in the medium is probably also the reason of the electrical stimulation of leucine and lysine efflux observed by Katz *et al.* (1969).

The protein synthesis is inhibited by glutamate (Takahashi & Akabene, 1960) and certain other amino acids (Orrego & Lipmann, 1967).

f. Acetylcholine effects: The potassium-induced decrease in glycine accumulation may be counteracted by acetylcholine (Nakazawa & Quastel, 1968b) which also enhances the incorporation of uridine into RNA (Prives & Quastel, 1969). The latter effect is absent in homogenates and requires the presence of both sodium and potassium.

g. Calcium and magnesium effects: The presence of calcium in the medium in minute concentrations is essential for a maximum release of acetylcholine both from ganglia (Harvey & McIntosh, 1940; Hutter & Kostial, 1954; Birks, 1963), from brain slices (McLennan & Elliott, 1950) and from the exposed cortex (Randic & Padjen, 1967), and it has been suggested (Hodgkin & Keynes, 1957; Shanes, 1958b, p. 263) that the calcium entry during the nerve impulse (Hodgkin & Keynes,

1957) triggers the acetylcholine release. Such a calcium dependent transmitter release would represent an analogy with the calcium entry into the secreting adrenal medulla (Douglas & Poisner, 1962) and the calcium activation of muscular contraction (IV B, 5a). Direct measurements of the calcium entry into the stimulated excised ganglion (Lipicky et al., 1963) have, however, failed to demonstrate any certain correlation between the uptake of calcium ion and the release of acetylcholine (cf. however also Katz & Miledi, 1965), and the calcium ion seems to play a dual role, since an increase of its concentration above 1 mM leads to an inhibition of the potassium-evoked output of acetylcholine (Mann et al., 1939; McLennan & Elliott, 1950; Birks, 1963). High concentrations of magnesium may have a similar effect (Mann et al., 1939).

Calcium is also required for a maximum stimulation of the release of norepinephrine (Baldessarini & Kopin, 1966) and of GABA (Katz et al., 1969; Srinivasan et al., 1969; Arnfred & Hertz, 1971), aspartate and glutamate (De Belleroche & Bradford, 1972) and may possibly be counteracted by magnesium.

Omission of calcium from the medium resembles excess potassium in decreasing the accumulation of tyrosine and glycine (Guroff et al., 1961; Abadom & Scholefield, 1962a; De Almeida et al., 1965). The simultaneous omission of magnesium causes a further decrease of the uptake (Abadom & Scholefield, 1962a). The content of glutamate may, in contrast, be increased (Takagaki et al., 1959; cf. however also Tsukada et al., 1963).

The incorporation of amino acids into protein may be decreased in calcium-free media and slightly increased in magnesium-free media (Mase et al., 1962). Huttunen (1969) observed, however, no effect of calcium on the incorporation of leucine into protein and of orotic acid into RNA, whereas the incorporation was reduced in magnesium-free media.

2. Metabolism

a. Potassium effects: After labelling with ^{14}C -glucose (which enters the different compartments equally) addition of excess potassium or of veratrine (Quastel, 1960) causes an increase of the rate of incorporation of radioactivity into glutamine, GABA and glutamate (Quastel, 1959; Kini & Quastel, 1959, 1960; Lahiri & Quastel, 1963; Machiyama et al., 1970), whereas the incorporation rate is reduced in potassium-free media (Quastel, 1960). The potassium-induced increase in glutamate and GABA formation is probably evoked by the increased turn-over rate in the tricarboxylic acid cycle(s) (Machiyama et al., 1970). In accordance with this concept, Machiyama et al. (1970) observed also a considerable stimulation of the incorporation of ^{14}C into aspartate in potassium-rich media, whereas

Quastel and coworkers found a reduced incorporation or no change (Quastel, 1959; Kini & Quastel, 1959; 1960; Gonda & Quastel, 1962a; Lahiri & Quastel, 1963). It is a further indication of an increased turnover of the tricarboxylic acid cycle(s) that the formation of radioactive carbon dioxide (table 6 in Quastel, 1959) and of labelled ninhydrin-negative degradation products (Potter & Van Harreveld, 1962) is enhanced by excess potassium after addition of ^{14}C labelled glutamate, and that the formation of GABA and aspartate from labelled glucose is enhanced by dinitrophenol (Gonda & Quastel, 1963; cf. also Smith and Moses, 1960), which conceivably could be the reason of the concomitant increase in the level of GABA (Cremer, 1964, 1967b). The formation of glutamine is, in contrast, not increased by dinitrophenol, and addition of this drug to a potassium-rich medium almost abolishes the incorporation of labelled glucose into glutamine (Kini & Quastel, 1959; Quastel, 1960; Gonda & Quastel, 1963). Fluoroacetate in concentrations evoking severe neurological disturbances without major effect on brain respiration, inhibits the increased formation of glutamine, which is evoked by addition of ammonium or excess potassium, but exerts no direct inhibition of the glutamine synthetase (Lahiri & Quastel, 1963; cf. also Benjamin & Quastel, 1972).

After addition of the radioactive compounds entering the pools unequally the compartmentation is more or less totally broken down, i.e. the relative specific activity of glutamine decreased by excess potassium (Berl et al., 1968, 1970b; Machiyama et al., 1970; Lund-Andersen et al., 1971). Conceivably this might either indicate a relative decrease of the flow of the precursor into the small pool (Berl et al., 1968) or an increase in the transfer of intermediates from the small to the large pool, reflected by the rapid release to potassium-rich media of small compartment intermediates (cf. Machiyama et al., 1970, and V B, 1c). It is in accordance with the latter concept that the metabolism of GABA, which mainly seems to be produced in the large pool (cf. V A, 2a) becomes relatively reduced in potassium-rich media leading to an increased GABA concentration (Machiyama et al., 1970), and that the formation of glutamine from glutamate (which predominantly seems to occur in the small pool) may be increased (table 3 in Berl et al., 1968; Machiyama et al., 1970; cf. however also table 5 in Lahiri & Quastel, 1963; Gonda & Quastel, 1963). An increased glutamine synthesis may, in turn, explain the tendency of moderately high concentrations of potassium to cause a lowering of the ammonium concentration in brain slices (Vrba, Folbergr & Kantourek, 1958; Rybová, 1959). On the other hand it is in support of a decreased operation of the small compartment that incorporation of radioactivity into glutamine from labelled acetate (small pool precursor) is reduced after addition of excess potassium (Berl et al., 1970b; H. Lund-Andersen, T. Arnfred & L. Hertz, unpublished results) or ammonium (Gonda & Quastel,

1966). The passage from the large to the small compartment via GABA (V A, 2a) is also not (or only slightly) increased by excess potassium (VI B, 1), and it seems unlikely that one of the pools during a prolonged period should lose intermediates to the other (cf. Van den Berg & Garfinkel, 1971).

b. Electrical stimulation: Application of electrical pulses resembles excess potassium in increasing the formation of amino acids from ^{14}C glucose (Orrego & Lipmann, 1967).

c. Calcium: The effects evoked by lack of calcium resemble those seen after addition of high concentrations of potassium, since the incorporation of radioactivity from ^{14}C labelled glucose into glutamine, GABA and glutamate becomes increased (Kini & Quastel, 1960) in calcium-deficient media. In the absence of calcium the compartmentation of glutamate metabolism is abolished after labeling with glutamate or aspartate, whereas it is only reduced if acetate is used as the ^{14}C source (Berl & Clarke, 1969; Berl, Clarke & Nicklas, 1970).

d. Sodium: The formation of GABA from L-glutamate occurs rapidly in a sodium-free medium (Takagaki *et al.*, 1959), whereas the metabolic conversion of ^{14}C labelled aspartate is reduced (Margolis & Lajtha, 1968).

C. DISCUSSION

It seems beyond doubt that the nitrogen metabolism to a large extent occurs in neurons as indicated by the good interspecies correlation between nerve cell density and the content and release of acetylcholine, and by the predominantly neuronal synthesis of protein. Alterations in RNA, and thus also in proteins during nervous function or disorders seem, however, to occur both in neurons and in neuroglia (e.g. Hydén & Egyhazi, 1963; Gomirato & Hydén, 1963; Hydén & Lange, 1966; Hydén, 1967a, c), and the dependence of protein synthesis in glia upon the presence of sodium may reflect the effect of sodium deficiency on glial metabolism (II B, 1b). Also the transmitters may conceivably have effects on glial function (e.g. the glial depolarization evoked by acetylcholine) and metabolism (e.g. the concept of neuroglial inactivation of transmitters). The importance of calcium for the transmitter release seems well established, and a corresponding role of calcium in the "cut off" of protein synthesis and resulting release of proteins has been suggested by Huttunen (1969). Excess potassium may conceivably increase the output of transmitters in a similar way as it affects the metabolism in muscle, where the potassium-induced depolarization may lead to an increase

in the intracellular concentration of free calcium. A raised calcium concentration may, in turn, exert a coupling action between depolarization and contraction which might explain the stimulation of oxygen uptake in muscle by high concentrations of potassium, sodium or calcium (cf. IV B, 5a). A similar coupling mechanism in transmitter release might lie behind the dependence of the release of e. g. acetylcholine upon the presence of both sodium (V B, 1b) and calcium (V B, 1g), and the ontogenetic difference between the potassium effect on acetylcholine release (V B, 1c) on one hand, and oxygen consumption (II B, 2a) and swelling (IV B, 2b) on the other, may reflect both different mechanisms of action and different cellular localization.

The potassium-induced release of glutamate resembles the potassium effects on oxygen consumption and swelling since both are absent neonatally, and the ontogenetic parallelism in the development of metabolic compartmentation in vivo (Fig. V, 2) and the potassium induced swelling in vitro (IV B, 2b) is striking. It may be symptomatic for the difficulties in relating metabolic events to certain anatomical structures, that the development of the compartmentation was interpreted to result from a differentiation and proliferation of neuronal processes (Patel & Balázs, 1970), whereas the concomitantly developing changes in water and ion metabolism and in the content of S-100 (Moore, 1969; Herschman et al., 1971) have been correlated with the proliferation and maturation of glia cells (cf. I B, 4). The ontogenetically simultaneous development of metabolic compartmentation and rapid formation (and large content) of glutamate supports the concept that the absence of compartmentation neonatally is due to the lack of operation of the large compartment (Patel & Balázs, 1970; Van den Berg, 1970a), but the role of glutamate, glutamine and GABA remains unknown. It seems, however, thoughtprovoking that all three amino acids are formed in large amounts during metabolic degradation in brain, that glutamate and GABA share several characteristics with genuine transmitters, and that glutamate is an anion which could function as the counterion in transport of cations. The metabolism of all three amino acids seems to be affected by excess potassium, but the flux via the GABA shunt is only slightly or not at all stimulated in potassium-rich media (VI B, 1), and the potassium-induced fluxes of glutamate between the tissue and the medium are far too small to account for a coupled transport of glutamate and potassium ions. This does, however, not necessarily exclude that such a transport might occur e. g. between different cells, and glutamate was suggested as a mediator during spreading depression (V B, 1c). The lack of effect by glutamate on potassium efflux from brain-cortex slices (IV B, 5d) seems, however, to be against the latter concept. The suggestion that both GABA and glutamine may function as links between the two compartments gives a special importance to the GABA shunt (VI A, 2; cf. Van den Berg, 1972) and indicates a more dy-

namic role of glutamine and a more active metabolism of this amino acid than generally envisaged (cf. Benjamin & Quastel, 1972; Watkins, 1972). So does the specific potassium- (or ammonium-) induced stimulation of glutamine formation and the inhibition of this process by fluoroacetate.

The reason that glutamate causes a respiratory stimulation in the absence of sodium (II B, 2a; VI B, 2e), and that certain amino acids, including glutamate, partially are able to replace sodium in the maintenance of the potassium content in the tissue (Takagaki et al., 1959; Margolis & Lajtha, 1968 - cf. IV B, 3b) remains obscure. The different effects of calcium lack on compartmentation when aspartate and acetate is used as the precursor may indicate the presence of more than one small compartment, and a compartmentation model based upon three compartments was suggested by Balazs et al. (1970). It seems, however, at present not possible with certainty to correlate any compartment with specific anatomical structures (cf. V A, 2a), and "the major direction for future work no doubt will be the correlation of metabolic compartments with cellular and subcellular structures" (Berl & Clarke, 1969).

Intermediary Metabolism

A. BRAIN METABOLISM

1. Evaluation of intermediary metabolism

The increase in energy metabolism evoked by the stimulatory agents gives per se no understanding of the biochemical point(s) of action of these agents. It is, however, well established that only certain substrates are able to maintain the oxygen uptake and its response to stimulation, the production of energy-rich phosphates and of acetylcholine, the active uptake of amino acids and the transport of monovalent cations into brain slices. Knowledge of the modifications of these parameters, which are evoked by changes of the substrates, e.g. from glucose via pyruvate to the tricarboxylic acid cycle intermediates may both suggest analogies and discrepancies between the different phenomena and the point(s) of action by the stimulatory agents. Indications in this direction may also be obtained from information of changes in the levels of metabolic intermediates caused by e.g. potassium or glutamate, changes in the quantitative importance of different metabolic pathways and ion effects on isolated enzyme systems.

2. In vivo

There is good evidence that the normal intact brain predominantly utilizes glucose as its substrate and source of energy (e.g. Kety, 1957; Balázs, 1970) though fatty acids probably may be metabolized neonatally (cf. Klee & Sokoloff, 1967) and after prolonged fasting (Owen, Morgan, Kemp, Sullivan, Herrera & Cahill, 1967). This does not imply that glucose exclusively or predominantly is metabolized along the glycolytic pathway and subsequent tricarboxylic acid cycle, since both the pentose-phosphate shunt and the GABA-shunt (Roberts, 1956) might contribute considerably to cerebral glucose metabolism. The GABA shunt has been found metabolically

active in vivo (Lajtha et al., 1959) and the pentosephosphate shunt has by one author (Moss, 1964) been reported to be a major pathway for cerebral glucose oxidation, but by other authors (e.g. Sacks, 1957) to be of practically no importance in vivo.

The distinction between a large and a small pool of certain metabolites (V A, 2a) does not per se give any information about flux magnitudes. Based upon data from different laboratories for several precursors in different animals, Garfinkel (1966) estimated that the fluxes are about equal in the two compartments, whereas Van den Berg and Garfinkel (1971) for the mouse brain deduced fluxes of 1.25 and 0.3 $\mu\text{moles/g wet wt. per min}$ (Fig. V, 1) in respectively the large and the small compartment, combined with a flux between the two compartments (mediated by GABA, respectively glutamine - cf. V A, 2a) of 0.14 $\mu\text{moles/g wet wt. per min}$. In the rat, the flux between the two compartments seems to be about half of this value (Dzubow & Garfinkel, 1970) possibly reflecting the differences in rate of oxygen consumption (II A, 1a; cf. also Fig. II, 1).

The amount of glucose consumed by the brain in vivo corresponds almost quantitatively to the production of CO_2 measured as oxygen consumption and assuming a RQ of 1.00 (Kety, 1965; cf. however also Sacks, 1969) indicating that the production of lactate is small under ordinary experimental conditions. It increases, however, greatly (from 6 to 400 $\mu\text{moles/g wet wt/hr}$) when convulsions are evoked (McIlwain, 1966, p. 81; cf. also Klein & Olsen, 1947).

3. In vitro

a. Pathways: Also during incubation of brain slices in vitro most of the energy production is derived from breakdown of glucose as reflected by a RQ relatively close to 1.0 (cf. VI A, 3c). The function of the pentosephosphate shunt in mammalian and frog brain tissue and retina during in vitro experiments has been reported by several authors (e.g. Cohen & Noell, 1960; Hotta, 1962; Reading, 1964; Nishimura & Kimura, 1965; De Piras & Zadunaisky, 1965). Also the GABA shunt has been found active in brain slices, but different estimates of its quantitative importance have been reported. The reasons for these discrepancies were discussed by Hammond, Julian, Machiyama & Balázs (1970), who found that the GABA flux in vitro accounts for about 8 % of the total flux through the tricarboxylic acid cycle (Balázs et al., 1970).

b. Substrates: Several substrates cause an initially high, but subsequently rapidly declining rate of oxygen uptake.

Table VI, 1

Rate of oxygen uptake (μ moles/g/hr)		Concentration of ~ P (μ moles/g)		K ⁺ concentration (μ moles/g)		Na ⁺ concentration (μ moles/g)		Potential (mV)
rest.	stim.	rest.	stim.	rest.	stim.	rest.	stim.	rec.
No substrate								
1)34 2)	1)32*	h						
40		c		16)26)		16)		
3)4)5)	5)			33-39		26* 24-25 g		21)
41-44	27*	g		17)3)18)		125		45 g
6)7)	6)			0.53-0.66 0.66* g		17)18)		
46-50	6	r		29-43		g 111-123		g
				19)27)		27)		
				8-36		r 110		
Glucose								
1)41 2)	1)81*	h						
65		c		16)		16)		
5)	5)			57		41* 48		21)
54	94*	g		3)17)18)		g 108		58 g
4)8)3)	8)4)3)			5) 1.96* g		17)18)		
56-68	94-111	g		3.01 15)		g 91-106		g
9)	9)			2.09-2.43		r		
70	185	g						
10)11)7)12)	11)10)12)			7)		27)22)		
90-111	150-183	r		2.01 1.31 r		74-78 99		r
Fructose								
4)56 7)12)		g		16)52		40* 47		g
86-112	179	r		1.55		r		
Pyruvate								
1)45 5)	1)63*	h						
57	5)			16)		16)		21)
7)12)	93*	g		53		35* 44		g 117
104-118	12)	7)		19)		133* 125		g 58 g
	168	r		r 21		r		

Lactate	1)48 4)60 12)105	1)57* 4)100 12)150	h g r	16)52	34* 43	g 16)110	129* 114	g 21)54	g
Citrate	1)50 4)5)58-66	1)42* 5)62*	h g	5)1.68	5)1.02*g				
α-ketoglutarate	5)70 10)40 7)74	5)66* 10)36	g r r	19)9 r 27)53		r 27)114		r	
Succinate	1)43 4)5)74-89 7)12)75-96	1)41* 5)79* 12)80	h g r	5)0.87 7)1.01	5)0.72*g 16)37 r	g 16)139	150* 147	g	
Fumarate	1)48 5)49 4)8)47-69 7)65	1)49* 5)38*	h g g	5)0.76 7)0.65	5)0.66*g 16)37 r	g 16)138	160* 162	g	
Malate	7)56		r	7)0.68	r				
Oxaloacetate	9)130 7)78	9)210	g r	16)52 r 27)74	38* 47	g 16)127 r 27)91	148* 143	g 21)54	g r
L-Glutamate	1)50 2)64 3)4)64-70 1)87 10)12)7) 63-77 13)111	1)58* 1)76* 10)12) 42-62 13)147	h c g g r r	14)0.1 7)0.90	g 16)52 18)3)48-57 r 19)14	g 16)125 g 18)110	144* 141	g 21)44	g g
GABA	2)64		c	19)12					
Succinic semialdehyde	2)77		c						
L-Glutamate and glucose	1)55 8)3) 76-86 1)83	1)85* 8)3) 129-141 1)84*	h g g	25)16) 56-57 18)3) 69-83 r 19)37	39* 48	g 100-115	127* 125	g 24)34	g g
	1)77)70-75 10)107 13)122	1)75* 10)109 13)197	r r r	7)1.03		r 18)102		r	

Table VI, 1. Rates of oxygen uptake, concentrations of energy-rich phosphates ($\sim P$), concentrations of potassium, concentrations of sodium and magnitudes of membrane potentials in brain slices during incubation in vitro. The rates of oxygen uptake and the concentrations of energy-rich phosphates are shown both under "resting" conditions and after stimulation with either high concentrations of potassium or electrical pulses. The latter mode of stimulation is indicated by *. The ionic concentrations are similarly shown both under "resting" conditions and after stimulation, but in addition also values obtained after recovery from stimulation are quoted.

The results shown were obtained using either human (h), cat (c), guinea-pig (g) or rat (r) brain cortical slices and the species is indicated in the table. When different groups of investigators reached almost identical values only the lower and upper limits of the range are presented, and the references (shown above or before the values) are given in such a sequence that the first reference refers to the lowest value and the last to the highest value quoted.

Results originate from:

1. Mc Ilwain, 1953a
2. McKhann et al., 1960
3. Takagaki et al., 1959¹⁾
4. Takagaki & Tsukada, 1957
5. Kratzing, 1953
6. Dickens & Greville, 1935
7. Abadom & Scholefield, 1962b
8. Tsukada et al., 1958a
9. Ito, 1960
10. Lipsett & Crescitelli, 1950
11. Hertz & Schou, 1962
12. Ghosh & Quastel, 1954
13. Quastel, 1959
14. Heald, 1960, p. 102
15. Heald, 1960, p. 79
16. Joanny & Hillman, 1963²⁾
17. Joanny & Hillman, 1964²⁾
18. Joanny et al., 1966²⁾
19. Bilodeau & Elliott, 1963¹⁾
20. Franck, 1970
21. Hillman, 1961
22. Lund-Andersen & Hertz, 1970, and unpublished results²⁾
23. Franck et al., 1968²⁾
24. Gibson & McIlwain, 1965
25. Harvey & McIlwain, 1968
26. McIlwain et al., 1961³⁾
27. L. Hertz, unpublished experiments²⁾

1) Ion concentration in $\mu\text{moles/g}$ initial wet wt.

2) Ion concentrations in $\mu\text{moles/g}$ final wet (incubated) wt.

3) Concentrations recalculated to $\mu\text{moles/g}$ final wet wt.

Absence of substrate: During incubation in media prepared without any substrate both the rate of oxygen uptake and the level of energy-rich phosphates are low (Dickens & Greville, 1935; McIlwain, 1951a, 1952a, 1953c; McIlwain & Gore, 1953; Kratzing, 1953; Takagaki & Tsukada, 1957; Heald, 1960; Woodman & McIlwain, 1961; Abadom & Scholefield, 1962b; Okamoto & Quastel, 1970a; - cf. table VI, 1) and no incorporation of ^{32}P into acid-soluble phosphates occurs (Findlay *et al.*, 1954b). The acetylcholine synthesis is decreased (Quastel, Tenenbaum & Wheatley, 1936; cf. also Kahlson & McIntosh, 1939), and the active uptake of amino acids is inhibited (Abadom & Scholefield, 1962b). The potassium concentration in the tissue declines drastically (Gardos, 1961; Bilodeau & Elliott, 1963; Joanny & Hillman, 1963, 1964; Joanny, Turc & Corriol, 1965; Joanny *et al.*, 1966), and the sodium concentration increases (Joanny & Hillman, 1963, 1964; Joanny *et al.*, 1966). The membrane potentials decrease only slightly in slices (Hillman, 1961) but very markedly in isolated cells (Hillman & Hydén, 1965a). The disappearance of the potentials in the slices becomes, however, complete and irreversible, if the tissue simultaneously is exposed to both anoxia and lack of substrate at the start of the incubation (Hillman, 1961).

Glucose, fructose, pyruvate and lactate: These four substrates all support identical, well-maintained rates of oxygen uptake during incubation in a "balanced" medium (e.g. McIlwain, 1953a; Ghosh & Quastel, 1954; Ito, 1960; table VI, 1). A high concentration (1.0-2.0 $\mu\text{moles/g}$ wet wt.) of energy rich phosphates is obtained with either glucose, fructose or pyruvate (McIlwain, 1952a; Heald, 1960, p. 79; Woodman & McIlwain, 1961; Abadom & Scholefield, 1962b; Okamoto & Quastel, 1970a).

Pyruvate and lactate may more or less effectively replace glucose in the synthesis of acetylcholine (Quastel *et al.*, 1936), in the maintenance of ^{32}P incorporation into pentose phosphates (Findlay *et al.*, 1953), and in the glutamate-induced accumulation of potassium (Turner *et al.*, 1950). All four substrates maintain some uptake of amino acids (Stern *et al.*, 1949; Guroff *et al.*, 1961; Johnstone & Scholefield, 1962). Almost the same concentrations of potassium are obtained in slices with either of these four substances (table VI, 1), but the sodium concentrations are lowest with glucose or lactate (Joanny & Hillman, 1963; Joanny, Corriol & Hillman, 1963), and a higher substrate concentration is required to keep the sodium concentration low than to keep the potassium concentration high (Joanny & Hillman, 1964). Both glucose, pyruvate and lactate maintain maximum membrane potentials (Hillman, 1961).

Citrate, α -ketoglutarate, succinate, fumarate and malate: According to Kratzing (1953), both citrate, α -ketoglutarate and succinate are able to sustain a normal

(or even an increased) rate of oxygen uptake, whereas the respiration is somewhat lower with fumarate as the substrate (table VI, 1). A high rate of oxygen uptake when succinate is used as the substrate has also been observed by McIlwain (1953a) and by Takagaki & Tsukada (1957), but other authors have observed a decreased rate of oxygen uptake with ketoglutarate (Lipsett & Crescitelli, 1950; Abadom & Scholefield, 1962b) or succinate (Ghosh & Quastel, 1954; Abadom & Scholefield, 1962b); Okamoto & Quastel, 1970a) as the substrate. This discrepancy may possibly be explained by a rapid respiratory decline which, however, may be counteracted by addition of small amounts of pyruvate (Ito, 1960; cf. VI A, 3c). According to Kerpel-Fronius & Hajos (1971) presynaptic mitochondria are unable to oxidize ketoglutarate and succinate, whereas perikaryal dendrites and glial mitochondria seem to have an intact tricarboxylic acid cycle; only the "non-axonal terminal", compartment should thus be able to carry a complete oxidation through.

The P/O ratio is reasonably high in brain mitochondria oxidizing ketoglutarate malate, fumarate or succinate (2.4-1.8 in the sequence mentioned), but smaller (1.3) when citrate is used (Brody & Bain, 1952; Heald, 1960; p. 83). The concentration of energy-rich phosphates in brain slices is, however, higher with citrate as the substrate, than without any substrate (Kratzing, 1953); ketoglutarate leads also to a high level of energy-rich phosphate, whereas fumarate causes no, and succinate and malate little increase above the level in a substrate-free medium (Kratzing, 1953; Woodman & McIlwain, 1961; Abadom & Scholefield, 1962b; Okamoto & Quastel, 1970a). These substrates are not able to sustain the active uptake of glutamate (Stern *et al.*, 1949) or the incorporation of ^{32}P into pentose phosphates (Findlay *et al.*, 1953), and succinate cannot replace glucose in the synthesis of acetylcholine (Quastel *et al.*, 1936).

The potassium concentration in the tissue (table VI, 1) may be maintained at a relatively high level with ketoglutarate as the substrate (Joanny *et al.*, 1963; L. Hertz, unpublished results; cf. however also Bilodeau & Elliott, 1963; Joanny *et al.*, 1966), whereas citrate, succinate, fumarate or malate lead to a potassium concentration which is similar to, or below that obtained without any substrate (Joanny & Hillman, 1963; Joanny *et al.*, 1963; Joanny *et al.*, 1966). With succinate, fumarate or malate as the substrate the sodium concentration becomes even higher than without any substrate (Joanny & Hillman, 1963; Joanny *et al.*, 1966), whereas ketoglutarate has no significant effect on the sodium level (Joanny *et al.*, 1966). Ketoglutarate does not share the ability of glutamate to evoke an increase in the potassium content (Termer *et al.*, 1950).

Oxaloacetate: Oxaloacetate differs from the above-mentioned substrates in maintaining a high rate of oxygen consumption during prolonged incubation (Ito, 1960 - cf. however also Abadom & Scholefield, 1962b). It gives rise to a P/O ratio

of 2.5 in brain mitochondria (Brody & Bain, 1952), leads to a reasonably high level of energy-rich phosphates (Woodman & McIlwain, 1961; Abadom & Scholefield, 1962b), maintains some active uptake (or metabolic formation) of glutamate (Stern et al., 1949; Guroff et al., 1961), sustains the polarization of nerve cells (Hillman, 1961) and maintains a high potassium concentration (table VI, 1) and an intermediate level of sodium (Joanny & Hillman, 1963; Joanny et al., 1963; L. Hertz, unpublished experiments). The presence of oxaloacetate together with glucose protects against anoxia (Phizackerly & Fixter, 1966).

Glutamate, glutamine and GABA: The use of glutamate as the only substrate may lead to a decreased rate of oxygen uptake (Lipsett & Crescitelli, 1950; Ghosh & Quastel, 1954; Quastel, 1962b). Other authors have, however, observed a high, often relatively well maintained respiratory rate (McIlwain, 1951c; Takagaki & Tsukada, 1957; McKhann et al., 1960), and a species difference seems to occur (McIlwain, 1953a). Both GABA and succinic semialdehyde are capable of supporting respiration in brain tissue preparations at approximately the same rate as glucose (Tsukada, Nagata & Takagaki, 1957; McKhann & Tower, 1959; Tsukada et al., 1960a; McKhann et al., 1960).

The P/O ratio in mitochondria metabolizing GABA, succinic semialdehyde or glutamate is high (2.4-3.0) (Brody & Bain, 1952; McKhann & Tower, 1959; McKhann et al., 1960). Nevertheless, the level of energy-rich phosphates (down to 0.1 μ moles/g wet weight) is significantly lower with glutamate as the substrate than without any substrate (McIlwain, 1951c, 1952a; cf. however also Abadom & Scholefield, 1962b), and the incorporation of ^{32}P into acid-insoluble phosphates is inhibited (Rossiter, 1955). The concentration of phosphocreatine is also low with GABA as the substrate (Woodman & McIlwain, 1961).

Other important functions are relatively well maintained with glutamate as the only substrate. The synthesis of acetylcholine is thus intermediate between that obtained with glucose and that seen without any substrate (Quastel et al., 1936), and some active accumulation of glutamate may occur (Takagaki et al., 1959; cf. however also Stern et al., 1949). The sodium concentration in the tissue is equal to or only little below that obtained in the absence of a substrate (Joanny & Hillman, 1963; Joanny et al., 1963). The potassium concentration is relatively high (Krebs & Eggleston, 1949; Takagaki et al., 1959; Cummins & McIlwain, 1961; Joanny & Hillman, 1963; Joanny et al., 1963; Joanny et al., 1966), though only little reaccumulation of potassium ions occurs after preincubation in the cold (Bilodeau & Elliott, 1963), and the membrane potential is identical to that observed in the absence of any substrate (Hillman, 1961). GABA does not share the ability to maintain a high concentration of potassium (Tsukada et al., 1960a).

Acetate: The compartmentation of glutamate metabolism with ^{14}C labelled acetate (V A, 2b) indicates that this substrate is utilized by brain tissue but the oxygen consumption with acetate as the sole substrate is lower than with glucose (Gonda & Quastel, 1966).

c. Intermediates: The pyruvate concentration in brain slices respiring with glucose as the substrate has been found to a little below $1\text{ }\mu\text{mol/g}$ wet wt. (Takagaki, 1968) and the importance of a pyruvate concentration above $5 \times 10^{-5}\text{ M}$ to maintain respiration was pointed out by Ito (1960). The other intermediates of aerobic glycolysis occur in smaller concentrations, but the lactate concentration in the tissue is relatively high (Takagaki, 1968). The rate of lactate production seems to be higher in brain slices than in the brain in vivo, and most authors reach estimates of about $25\text{ }\mu\text{moles/g}$ wet wt. per hour (e.g. Elliott & Henderson, 1948; Dixon, 1949; McIlwain, 1953a, c; Thomas & McIlwain, 1956). Also the total absence of lactate production has, however, been reported (e.g. Dickens & Greville, 1935). The reason of this discrepancy probably is that the aerobic glycolysis declines rapidly with time (Elliott & Henderson, 1948; McIlwain, 1953b; Cremer, 1967a; cf. however also Kimura, 1940).

Together the production of lactate and the respiration account relatively well for the consumption of glucose (McIlwain, Anguiano & Cheshire, 1952). The presence of $5\text{ }\%$ CO_2 has been reported to increase the glycolytic rate (Craig, 1944) but this was not confirmed by Birmingham & Elliott (1948). Also suspensions of homogenized brain tissue are capable of rather intensive aerobic glycolysis (Reiner, 1947).

Anaerobiosis increases the rate of the glycolysis in most tissues, including brain (the "Pasteur effect"). The values given in the literature for anaerobic lactate production in brain slices vary from about $40\text{ }\mu\text{moles/g}$ wet wt. per hour in the rat (Elliott & Henderson, 1948) to $100\text{--}200\text{ }\mu\text{moles/g}$ wet wt. per hour in man and in the rabbit (Elliott & Penfield, 1948; Dixon, 1949). This means, that the glycolytic rate is higher in species of bigger body size, i.e. the correlation is the reverse of that found with respect to oxygen uptake (cf. Elliott, 1952, 1957).

Homogenization may increase the rate of anaerobic lactate production, but it varies considerably with the dilution of the preparations (Elliott & Henry, 1946), and with the ionic composition of the medium (cf. VI B, 3a-c). Microdissected neurons and glia cells from the rabbit show an anaerobic glycolysis corresponding to about $8 \times 10^{-4}\text{ }\mu\text{l CO}_2/\text{hr}$ per sample ($20 \times 10^{-9}\text{ g}$ dry wt.) or approximately $200\text{ }\mu\text{moles/g}$ wet wt. (Hamberger & Hydén, 1963), i.e. the same value as that observed with slices.

The pronounced decline with time in respiratory rate when succinate is used

as the substrate is accompanied by an accumulation of intermediates from the tricarboxylic acid cycle (Ito, 1960).

The production of carbon dioxide in brain slices is almost equal to the consumption of oxygen, giving a RQ close to 1.00 (e.g. Dickens & Simer, 1931; Elliott, Greig & Benoy, 1937; Elliott & Penfield, 1948; Nishimura & Kimura, 1965). After addition of labelled glucose the respiratory $^{14}\text{CO}_2$ production increases only slowly, indicating that the equilibration of the labelled metabolites with the pools requires appreciable time (Beloff-Chain *et al.*, 1955; Cremer, 1967a; Balazs *et al.*, 1970). $^{14}\text{CO}_2$ is also formed from labelled acetate (Beloff-Chain, Catanzaro, Chain, Longinotti, Masi & Pocchiari, 1962; Gonda & Quastel, 1966; Chan & Quastel, 1967, 1970), and the finding that 5-6 % of the CO_2 production in a medium containing glucose and labelled acetate originates from the latter (Lindbohm & Wallgren, 1966) must thus indicate that the degradation of acetate accounts for at least 5-6 % of the total metabolism.

B. ION EFFECTS ON BRAIN METABOLISM

1. Pathways

The turnover via the pentose phosphate shunt is not increased during "stimulation" with electrical pulses (O'Neill, Simon & Shreeve, 1965) or high concentrations of potassium (De Piras & Zadunaisky, 1965; Kozawa, 1961), whereas the tricarboxylic acid cycle (and aerobic glycolysis) operates at an increased rate under the influence of excess potassium (Machiyama *et al.*, 1970; cf. also Kimura & Niwa, 1953). If the passage through the GABA shunt is increased in potassium-rich media (Machiyama *et al.*, 1967) the absolute increase is only small and the flux along this pathway decreases from 8% to 3-5% of the flux via the tricarboxylic acid cycle (Machiyama *et al.*, 1970).

2. Substrates

a. Absence of substrate: In the absence of a substrate neither high concentrations of potassium, nor electrical pulses increase the rate of oxygen uptake (Dickens & Greville, 1935; Kimura & Ito, 1952; Kratzing, 1953; McIlwain, 1953a; Vrba & Folbergr, 1958). Application of pulses under these conditions even abolishes the subsequent increase of respiration (but not the increase of aerobic glycolysis (VI B, 3b)) when glucose subsequently is added and pulses applied (McIlwain & Gore, 1953). In spite of the low potassium concentration, application of electri-

cal pulses leads to a further decrease (Cummins & McIlwain, 1961; Joanny & Hillman, 1963, 1964), but no reaccumulation occurs after the termination of the pulses (Joanny & Hillman, 1963). Omission of sodium in the absence of a substrate causes a further reduction in the low rate of oxygen-uptake (Takagaki & Tsukada, 1957).

b. Glucose, fructose, pyruvate and lactate: In media containing either glucose, fructose, pyruvate or lactate as the substrate the respiration is stimulated by high (20-100 mM) concentrations of potassium (e. g. Dickens & Greville, 1935; Dixon & Holmes, 1935; (Lipsett & Crescitelli, 1950; Ghosh & Quastel, 1954 - cf. also table VI, 1). Also the excitability to electrical pulses is maintained, though fructose is required in a higher concentration (10 mM) than glucose (McIlwain, 1953c). The simultaneous presence of glucose and trace amounts of pyruvate has been reported to reduce the potassium-induced stimulation of oxygen consumption (Lipsett & Crescitelli, 1950). With fructose or lactate as the substrate the oxygen consumption is decreased when all sodium is replaced with choline, whereas this substitution is of little effect when glucose is oxidized (cf. II B, 1a), and may lead to an increased metabolic rate with pyruvate as the substrate (Takagaki & Tsukada, 1957).

With each of these compounds as the substrate, application of electrical pulses leads to a potassium loss (and sodium gain) followed by a reaccumulation (and re-extrusion) after the termination of the stimulation (Joanny & Hillman, 1963). Lactate or pyruvate may replace glucose in the potassium accumulation evoked by glutamate (Terner et al., 1950).

c. Citrate, α -ketoglutarate, succinate and fumarate: The rate of oxygen uptake by brain slices incubated in media with citrate (Kratzing, 1953; McIlwain, 1953a), α -ketoglutarate (Lipsett & Crescitelli, 1950; Kratzing, 1953), succinate (Kratzing, 1953; McIlwain, 1953a, Ghosh & Quastel, 1954) or fumarate (Kratzing, 1953; McIlwain, 1953a) as the substrate is unaffected, or slightly inhibited by the addition of high concentrations of potassium and decreased in sodium-free media (Takagaki & Tsukada, 1957). If a minimum concentration of pyruvate (0.5 mM) is present together with one of these substrates, a "normal" response to the high concentrations of potassium may, however, be obtained, though the amount of pyruvate remains unchanged (Ito, 1960).

Application of electrical pulses leads to a decreased potassium content when either succinate or fumarate is oxidized, but no reaccumulation occurs (Joanny & Hillman, 1963). Nevertheless the concentration of energy-rich phosphates becomes further reduced (cf. table VI, 1).

d. Oxaloacetate: Oxaloacetate differs from the other tricarboxylic acid cycle intermediates in giving rise to a stimulation of the oxygen uptake with excess potassium (Ito, 1960) or with veratrine (Wollenberger, 1955a) and sustaining an accumulation of potassium after the loss evoked by electrical stimulation (Joanny & Hillman, 1963). The ability of oxaloacetate to maintain both a high respiratory rate (VI A, 3b) and the responsiveness to stimulation has been explained by a spontaneous decomposition of oxaloacetate into pyruvate and carbon dioxide (Ito, 1960).

e. Glutamate and GABA: Lipsett & Crescitelli (1950) and Ghosh & Quastel (1954), who found a low resting rate of oxygen consumption with glutamate as the substrate, observed also no stimulation when excess potassium was added. A relatively large response to high concentrations of potassium or electrical pulses (table VI, 1) was, however, observed by Tsukada et al. (1958a), Quastel (1959) and McIlwain (1953a). When both glucose and glutamate are present, some potassium-induced stimulation of the oxygen consumption has almost invariably been reported (McIlwain, 1953a; Tsukada et al., 1958a; Quastel, 1959; Takagaki et al., 1959; cf., however also Lipsett & Crescitelli, 1950). A high rate of oxygen uptake may be obtained in sodium-free media if both glucose and glutamate are present (Takagaki et al., 1959), but sodium deficiency leads to a low respiratory rate with glutamate as the only substrate (Takagaki & Tsukada, 1957).

Also with glutamate as the substrate, application of electrical pulses leads to a decrease in the potassium concentration. After the termination of the stimulation some reaccumulation occurs both in the presence and in the absence of glucose (Cummins & McIlwain, 1961; Joanny & Hillman, 1963). During incubation in media containing both glucose and glutamate, addition of potassium increases the rate constant of the potassium efflux from 0.037 min^{-1} to 0.106 min^{-1} (L. Hertz, unpublished experiments). This increase is only slightly smaller than that observed in a medium without glutamate (cf. IV B, 5a).

f. Acetate: Addition of excess potassium evokes no increase of the oxygen uptake with acetate as the only substrate, but the addition has the usual stimulatory effect if both acetate and glucose are present (Gonda & Quastel, 1966).

3. Intermediates

a. Sodium effects: Absence of sodium leads to an increase in the rate of aerobic lactate production by brain slices (Takagaki & Tsukada, 1957; Takagaki et al., 1959; Ruscak & Whittam, 1967; cf. however also Gore & McIlwain, 1952). The

formation of $^{14}\text{CO}_2$ from labelled acetate is reduced both in sodium-free media (Gonda & Quastel, 1966), and in media containing excess sodium (Chan & Quastel, 1970). The potassium-induced increase of aerobic lactate production (Takagaki & Tsukada, 1957; Ruscák & Whittam, 1967; VI B, 3b) and acetate metabolism (Gonda & Quastel, 1966) in brain-cortex slices requires the presence of sodium. No sodium is, however, necessary for the potassium-induced stimulation of glycolysis in muscle (Kaye & Mommaerts, 1960), but an increase of the sodium concentration leads *per se* to a rise of the aerobic lactate production (Muller, 1962; Clausen, 1965, 1968). The anaerobic glycolysis in brain slices is inhibited by the presence of sodium (Racker & Krimsky, 1945; Utter, 1950; Racker, 1952).

b. Potassium, glutamate and electrical stimulation: A considerable increase in the aerobic glycolysis by brain-cortex slices is evoked by high concentrations of potassium, rubidium, cesium or ammonium. A maximum response is obtained with e.g. 50-100 mM potassium (Ashford & Dixon, 1935; Kimura, 1937; Dixon, 1949; Gore & McIlwain, 1952) and the threshold concentration is about 20 mM (Takagaki, 1968, 1972). The stimulation requires the presence of sodium (cf. VI B, 3a), and is lost after homogenization (Kimura, 1937). Also omission of potassium from the incubation medium may, however, cause a stimulation of the aerobic lactate production (Dickens & Greville, 1935; Takagaki *et al.*, 1959). Addition of D- or L-glutamate causes a similar augmentation of the aerobic glycolysis (Weil-Malherbe, 1938; Woodman & McIlwain, 1961). This stimulation requires the presence of both potassium (Takagaki *et al.*, 1959; Takagaki, 1972) and sodium, and in sodium-deficient media the presence of glutamate causes, in contrast an inhibition of the lactate accumulation (Takagaki & Tsukada, 1957; Takagaki *et al.*, 1959). Also addition of GABA may lead to an increased aerobic glycolysis (Ruscák, Macejová & Ruscáková, 1964).

The aerobic lactate production is increased during (McIlwain *et al.*, 1952) and immediately after (McIlwain & Tresize, 1956) application of electrical pulses. Certain drugs (e.g. veratrine) have a similar effect. The stimulation of aerobic glycolysis by veratrine requires the presence of potassium (Wollenberger, 1955a), whereas no correlation was found between potassium content (in the medium or in the slices) and lactate production during application of electrical pulses (Cummins & McIlwain, 1961). This may, however, be related to the high rate of anaerobic glycolysis during incubation in potassium deficient media even without stimulation. Application of electrical pulses in the absence of a substrate does not prevent a subsequent stimulation of glycolysis by electrical pulses in the presence of glucose (McIlwain & Gore, 1953), though it does inhibit the corresponding stimulation of oxygen uptake (VI B, 2a).

The potassium induced stimulation of aerobic glycolysis is probably due to a

direct (VI B, 4a) or indirect (via ATP) effect on several enzymes including the pyruvate kinase (Takagaki, 1968), the phosphofructokinase and the glyceraldehyde-3-phosphatedehydrogenase (Takagaki, 1972) which belong to the enzymes that may control glycolysis in brain slices (Rolleston & Newsholme, 1967). Addition of glutamate (or lack of calcium) differ from excess potassium in their effect on the concentrations of certain glycolytic intermediates (Rolleston & Newsholme, 1967 ; Takagaki, 1968, 1971, 1972) and the over-all stimulation of glycolysis may be most directly correlated with the effect on the glyceraldehyde-3-phosphatedehydrogenase (Takagaki, 1972). This effect is probably mediated by the lowered concentration of ATP (III B, 2a) which in turn may be evoked by the increase in extracellular potassium concentration common to these stimulatory procedures (II C). The levels of glycolytic intermediates may, in addition, be influenced by the intracellular potassium concentration, which is increased by high potassium concentrations in the medium, but lowered by glutamate, electrical stimulation and calcium lack (IV B, 3b). Analogously, excess potassium and electrical stimulation have been found to affect the oxidation-reduction state of the respiratory chain differently (Cummins & Bull, 1971).

In contrast to potassium effects on aerobic glycolysis and on oxygen uptake, high concentrations of potassium cause a decrease in the anaerobic glycolysis. In potassium-rich media the aerobic glycolysis becomes accordingly greater than the anaerobic glycolysis. This "reversal of the Pasteur effect" has been observed by several investigators (e.g. Ashford & Dixon, 1935; Dickens & Greville, 1935; Dixon, 1949) but its mechanism is unknown (cf. also Elliott & Bilodeau, 1962). The potassium-induced decrease in anaerobic glycolysis may be counteracted by morphine, ethanol or barbiturates (Takemori, 1964). A similar inhibition by glutamate may be reversed by pyruvate (Weil-Malherbe, 1938). Also veratrine (Wollenberger, 1955a) or electrical pulses cause an inhibition, whereas dinitrocresol has no effect on the anaerobic glycolysis (McIlwain, 1956a). Addition of 40-100 mM sodium causes no, or only a very slight inhibition (Ashford & Dixon, 1935; Dickens & Greville, 1935; Dixon, 1949).

In muscle, high concentrations of potassium or electrical stimulation lead to an increase in the rate of both aerobic and anaerobic lactate production (e.g. Hegnauer et al., 1934; Kaye & Mommaerts, 1960; Kratzing, 1953; Muller & Simon, 1960). The presence of calcium but not of sodium is essential for the response (Kaye & Mommaerts, 1960).

The formation of $^{14}\text{CO}_2$ from labelled pyruvate or glucose is increased by high concentrations of potassium, by application of electrical pulses or addition of dinotrophenol (Quastel, 1959; Gonda & Quastel, 1962a; De Piras & Zadunaisky, 1965; O'Neill et al., 1965), and the output of labelled CO_2 from glucose seems to be more enhanced than the oxygen consumption (Hoskin, 1960; Cremer, 1967a,

Machiyama et al., 1970; cf. however also Gainer, Allweis & Chaikoff, 1962). A low RQ has, however, been reported during the early part of the potassium-induced stimulation (Nishimura & Kimura, 1965).

The production of $^{14}\text{CO}_2$ from labelled succinate is not increased by excess potassium (Gonda & Quastel, 1962a), whereas that from glutamate (Quastel, 1959, 1962a) or acetate (Gonda & Quastel, 1966) is raised both in the presence and in the absence of glucose. Application of electrical pulses causes a distinct reduction of the formation of CO_2 from labelled acetate (Gonda & Quastel, 1966; Lindbohm & Wallgren, 1966; Chan & Quastel, 1967) which seems to be related to the increase in the intracellular sodium concentration (Chan & Quastel, 1967, 1970) and is prevented by tetrodotoxin (Chan & Quastel, 1967, 1970).

c. Calcium and magnesium: The rate of aerobic glycolysis is increased when calcium is omitted from the medium, and the simultaneous absence of both calcium and potassium leads to a high, but rapidly declining, rate of lactate production (Dickens & Greville, 1935; Takagaki et al., 1959). An increase of the magnesium concentration of a magnitude which exerts no major inhibition of the stimulated oxygen consumption, may completely inhibit the electrically induced increase in lactate production (Gore & McIlwain, 1952).

Absence of calcium leads to a reduced entrance of acetate into brain cortex slices (Berl et al., 1970b), and to a decreased anaerobic glycolysis (Quastel & Wheatley, 1937; Quastel, 1962a, p. 234). The maximum glycolytic rate is obtained with about 4 mM calcium in the medium, but pyruvate and some organic bases (e.g. pyridine) are able to replace calcium (Adams & Quastel, 1956).

4. Potassium-activated enzyme reactions

a. Pyruvate kinase: Several enzymatic reactions are activated by monovalent cations (cf. Evans & Sorger, 1966). Among these is the reaction:



This process is catalyzed by the pyruvate kinase which has an absolute requirement for potassium (Boyer, Lardy & Phillips, 1942, 1943) and the optimum potassium concentration is about 0.1 M (Kachmar & Boyer, 1953; Melchior, 1965). NH_4^+ or Rb^+ may replace K^+ but Li^+ has no such effect (Kachmar & Boyer, 1953), and the pyruvate kinase is not (or only very slightly) activated by sodium, which in contrast, inhibits the reaction competitively (Utter, 1950, Kachmar & Boyer, 1953).

b. Acetylcoenzyme A synthetase: The formation of acetylcoenzyme A from acetate is stimulated by excess potassium and inhibited by sodium (Von Korff, 1953; Evans, Clark & Russell, 1964; Webster, 1966), and Quastel and coworkers have pointed out that also the rate of conversion of pyruvate to acetylcoenzyme A conceivably might be stimulated in media containing high concentrations of potassium (e.g. Kini & Quastel, 1959, 1960; Quastel, 1960, 1962a; cf. also Bilodeau & Elliott, 1963).

c. $\text{Na}^+ - \text{K}^+ - \text{ATPase}$: It has repeatedly been suggested that the potassium-induced stimulation of oxygen consumption might be a metabolic manifestation of an increased breakdown of ATP by $\text{Na}^+ - \text{K}^+ - \text{ATPases}$ (e.g. Minakami, Kakinuma & Yoshikawa, 1963; Henn *et al.*, 1972) or related enzymes (Hertz & Schou, 1962; cf. also II B, 2a). The activity of this enzyme is low in the absence of sodium and potassium. Addition of sodium alone, but not of potassium alone, causes a slight stimulation, whereas the simultaneous presence of both ions increases the activity considerably (Skou, 1957, 1960, 1962; cf. also Post, Merritt, Kinsolving & Albright, 1960). Maximum stimulation of enzyme preparations from the brain is obtained with 10-20 mM potassium (Fig. VI, 1; Samson & Quinn, 1967; Henn *et al.*, 1972). Potassium may be replaced by several other ions (e.g. NH_4^+ , Ca^{++} , Rb^+ , Li^+), but the sodium effect on the enzyme is rather specific, since only lithium is capable of causing some stimulation in the total absence of sodium (Skou, 1957; Bader & Sen, 1966; cf. Whittam, 1962a). Excessively high concentrations of either sodium (Post *et al.*, 1960; Whittam & Ager, 1962; Priestland & Whittam, 1968) or potassium (Skou, 1957, 1962; Ahmed, Judah & Scholefield, 1966) cause an inhibition of the enzyme.

Studies on neurons or glial cell samples obtained by microdissection (Cummins & Hydén, 1962) or gradient centrifugation (Henn *et al.*, 1972; Medzihradsky, Sellinger, Nandhasri & Santiago, 1972) have shown that the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ has a predominantly neuroglial localization. Corresponding investigations of microsamples from different layers of the cortex were, however, interpreted to show a localization in the plexi of dendrites and axons (Hess, Schneider, Warnoch & Pope, 1963; Lewin & Hess, 1964). Also nerve ending particles seem to possess a potent $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ (Hosie, 1965; Kurokawa, Sakamoto & Kato, 1965; Bradford, Brownlow & Gammach, 1966; Whittaker, 1969), and histochemical studies have shown ATPase activity between individual cells (I B, 2, cf. however also Mc D. Torney, 1966).

The $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity is much lower in new-born rats than in the adult animals and the increase in the activity seems to occur specially fast during the first 2-3 weeks after birth (Fig. VI, 2; Samson & Quinn, 1967; Abdel-Latif, Brody & Ramahi, 1967; Medzihradsky *et al.*, 1972), i.e. probably also

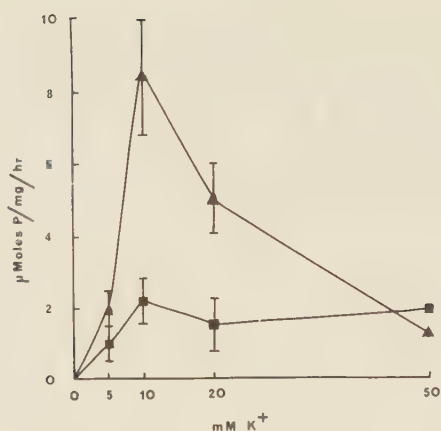


Fig. VI, 1. Specific activity of neuronal and glial Na⁺-K⁺-ATPase as a function of the external potassium concentration at unaltered sodium concentration. The activities (in μmoles phosphate liberated per mg per hr) are expressed as the increments of activity due to addition of sodium and potassium. Both the glial (▲) and neuronal (■) enzymes were incubated in media containing 75 mM Na⁺, 50 mM Tris-HCl at a pH 7.4 and 5 mM Mg⁺⁺, at 37° for 30 minutes. S.D. values are given as vertical bars. From Henn *et al.*, 1972.

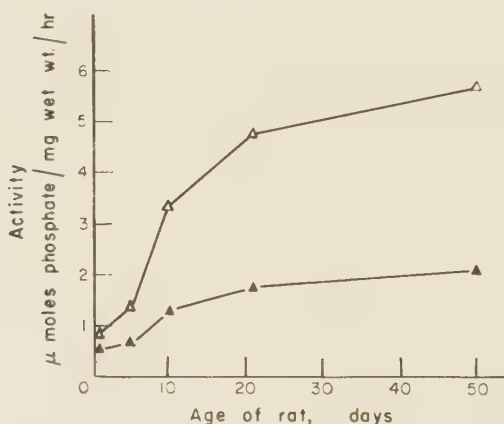


Fig. VI, 2. Specific activity of the Na⁺-K⁺-ATPase in rat brain homogenates exposed to 100 mM Na⁺ and 20 mM K⁺ in the absence (Δ), and presence (▲) of 5 x 10⁻⁴ M ouabain as a function of the age of the animals. Each point is an average of 20 experiments, and values are expressed in terms of wet weight of original brain. From Samson & Quinn, 1967.

somewhat before the onset of the potassium-induced increase in oxygen consumption (II B, 2a) and swelling (IV B, 2b). In the chicken a corresponding increase may be observed before hatching (Bignami, Palladini & Venturini, 1966; Zaheer, Iqbal & Talwar, 1968).

The activity of the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ is inhibited by ouabain (e.g. Post *et al.*, 1960; Skou, 1960; 1962; Schwartz, Bachelard & McIlwain, 1962; Gibbs, Roddy & Titus, 1965; Swanson & McIlwain, 1965; Israel & Titus, 1967; Henn *et al.*, 1972). Nevertheless, application of ouabain in concentrations above 10^{-6} M to brain slices (but not to homogenates (Wollenberger, 1947)) may lead to a transient stimulation in the rate of oxygen uptake which often is followed by a fast decline (Wollenberger, 1947; Joanny & Corrioll, 1964; Swanson & McIlwain, 1965; cf. however also Gonda & Quastel, 1962b; Quastel, 1965). The presence of calcium seems to be essential for the response (Schwartz, 1962; Whittam, Blond & Ruscák, 1965; Bourke & Tower, 1966b), and in the absence of this ion a decreased oxygen consumption is evoked (Whittam, 1961; 1962a). An inhibition of the potassium induced or the electrical stimulation of oxygen uptake by ouabain has been reported by Minakami *et al.*, (1963), Wallgren (1963a) and De Piras & Zadunaisky (1965), whereas Gonda & Quastel (1962b) found the same inhibition of the resting and the stimulated oxygen consumption, and Bourke & Tower (1966b) observed an enhancement of the potassium-induced stimulation, when a sub-maximum concentration of potassium was added to a medium containing 10^{-5} M ouabain.

A slight, but significant decrease of ATP and creatine phosphate concentrations has been observed after addition of ouabain (Wollenberger & Wahler, 1956; Yoshida *et al.*, 1963a; Rose, 1965a; Quastel, 1965; Rolleston & Newsholme, 1966), and the potassium concentration is low and the sodium and chloride concentrations increased in brain slices exposed to at least 10^{-6} M ouabain (Gardos, 1960; Whittam, 1962a; Schwartz, 1962; Joanny & Corriol, 1964; Swanson & McIlwain, 1965; Bourke & Tower, 1966b; Stahl & Swanson, 1971). Intracranial injection of ouabain or addition of this drug to cultures from the dorsal root ganglion leads to a rapid and very pronounced vacuolization of the glia cells, whereas the neurons remain largely intact (Fig. VI, 3; Bignami & Palladini, 1965, 1966; Stefanelli, Palladini & Iearadi 1965). Intracranial injection of ouabain into rats leads to motor hyperactivity, convulsions, and finally death (Bignami & Palladini, 1966).

Also ethanol may in pharmacologically active concentrations inhibit the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$, and the accumulation of potassium in several tissues including cerebral cortex (Järnefelt, 1961; Israel-Jacard & Kalant, 1965; Israel *et al.*, 1966). Relatively low concentrations of ethanol cause a slight increase of the resting rate of oxygen uptake (Fuhrman & Field, 1948; Ghosh & Quastel, 1954; Sutherland, Hine & Burbridge, 1956; Beer & Quastel, 1958; Wallgren & Kulonen, 1960), but an inhibition of the stimulated respiration (e.g. Ghosh & Quastel, 1954; Beer

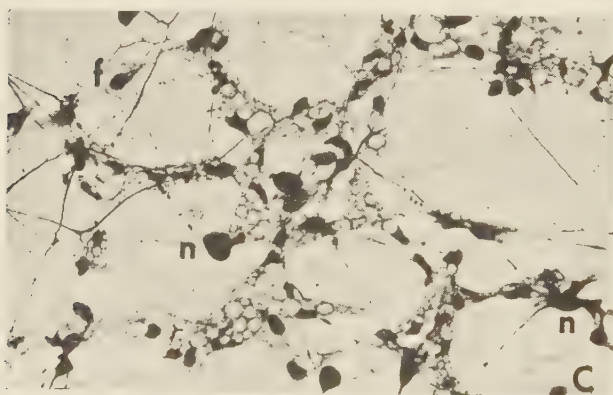
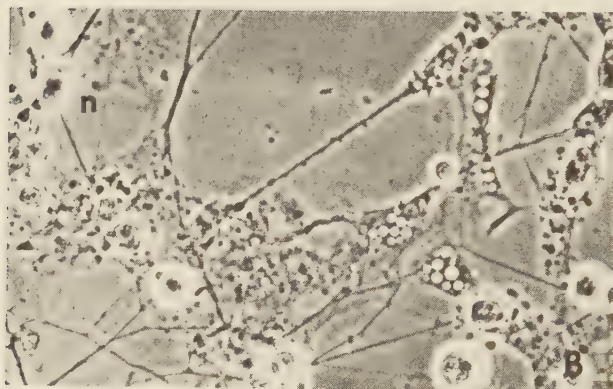
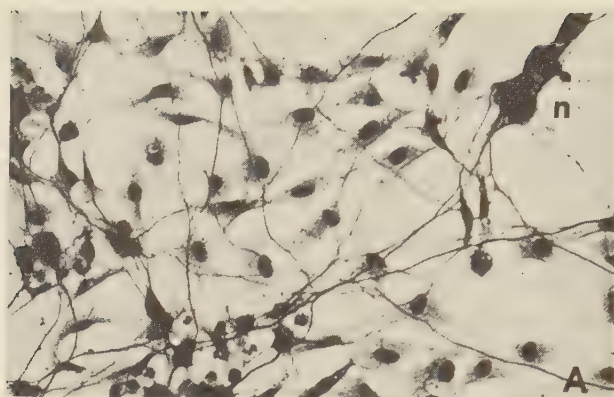


Fig. VI, 3. Cultures of dorsal root ganglion from the 11-12-day-old chick embryo. a: Fixed after 36 hrs of culture and Bodian stained, b: Treated with 1×10^{-4} M ouabain after 24 hrs of culture and observed in phase contrast microscope after 12 hrs of treatment; c. treated like b, but fixated and Bodian stained after 40 hrs of treatment. In b and c the glial cells show pronounced vacuolization with no effect on nerve cells (n) and typical fibroblasts (f). From Stefanelli et al., 1965.

& Quastel, 1958; Wallgren & Kulonen, 1960; Majchrowicz, 1965; cf. however also Sutherland et al., 1956). In electrically stimulated brain slices ethanol causes an increase in the concentration of GABA, glutamic acid and aspartic acid but a decrease in the glutamine level (Häkkinen, Kulonen & Wallgren, 1963).

C. DISCUSSION

No good concordance is found between the ability of different tricarboxylic acid cycle intermediates to maintain the oxygen uptake, the metabolic reactivity to stimulation, a large content of energy-rich phosphates, a high potassium concentration, a low sodium concentration and membrane polarization. Succinate, fumarate and malate maintain all functions poorly, and citrate seems only able to secure a rather high concentration of energy-rich phosphates. Ketoglutarate and oxaloacetate give rise to almost identical P/O values, relatively high concentrations of energy-rich phosphates and some accumulation of potassium. However, only the use of the latter of these substrates leads to the same potassium concentration as glucose, to an uptake (or formation) of glutamate, to a well-maintained rate of oxygen uptake which is stimulated by excess potassium and to some, though far from optimum, extrusion of sodium. Glutamate resembles ketoglutarate and oxaloacetate in securing a high P/O ratio and a high concentration of potassium, but the use of glutamate as the only substrate leads to a low concentration of energy-rich phosphates and to a decrease in membrane potential. These differences in substrate requirement support the concept that no close association is found between net transport of sodium and potassium or between average potassium content and polarization (cf. IV C). The finding that oxaloacetate as the substrate gives a "normal" potassium concentration in spite of an abnormally high sodium concentration is not compatible with both the concept that the membranes are more permeable to potassium than to sodium and that potassium accumulation is secondary to, or at least coupled with sodium extrusion.

That the ability of a substrate to maintain oxidative phosphorylation in brain mitochondria not necessarily implies that the presence of this substrate leads to a high level of energy-rich phosphates in brain slices might be due to lack of penetration into the slices (cf. Quastel, 1962b) or at least into some of the compartments. It is, however, against this concept that the rate of oxygen uptake may be relatively high with most of the substrates used. The reason of the very low level of energy-rich phosphates with glutamate as the substrate may rather be, that this amino acid acts as a "stimulating" ion, i. e., like high concentrations of potassium may stimulate some energy-requiring processes (cf. II B, 2a; III B, 6; IV B, 2c; IV B, 3a-b; IV B, 4c; V B, 1e and VI B, 3b). A similar

effect seems to be exerted by electrical pulses in the presence of all the substrates used as indicated by the lowering of both potassium concentrations and levels of energy-rich phosphates. An accumulation of potassium after the stimulation does, however, only occur with glucose, fructose, pyruvate, lactate or oxaloacetate (and to some extent glutamate), i. e. exactly the same substrates that maintain the potassium-induced stimulation of respiration. This seems to support the concept that the increased metabolism reflects an effect on ion distribution. Taken together with the general regulatory role of adenine nucleotides for metabolism in biological systems and the potassium-induced decrease in the level of energy-rich phosphates (III B, 2a) this may suggest that a major effect by potassium is exerted on the $\text{Na}^+\text{-K}^+\text{-ATPase}$ or a related enzyme (II, C). The $\text{Na}^+\text{-K}^+\text{-ATPase}$ shows quantitatively the same ionic dependance as the potassium-induced increase of the oxygen uptake by brain slices (cf. II B, 2a), and also the requirement for both sodium and potassium to cause a breakdown of ATP (III B, 2a), is in keeping with the function of a $\text{Na}^+\text{-K}^+\text{-ATPase}$. The profound influence by ouabain supports the concept of a crucial importance of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ (or a related enzyme) in brain metabolism, but the complex response complicates the use of this drug as a tool for qualitative and quantitative estimates of ion effects on the metabolic degradation in brain. In view of these indications of a correlation between the $\text{Na}^+\text{-K}^+\text{-ATPase}$ and the potassium effects on brain metabolism it may seem peculiar that ontogenetic differences seem to exist (VI B, 4c) and that the potassium concentrations required for maximum stimulation of the enzyme are about 20 mM, whereas higher potassium concentrations are needed to affect metabolism in intact cells. These discrepancies might conceivably indicate the presence of two independent enzyme systems in brain cortex of which one is reflected by the potassium activation of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ and the other by the potassium effects on energy metabolism in intact cells (cf. IV C). This concept may be supported by the evidence that an external potassium concentration of about 20 mM seems to stimulate an active potassium-sodium exchange, whereas higher concentrations are required to induce an uptake of potassium and chloride ions without effects on sodium transport (IV B, 5a). The latter mechanism seems to be localized in glial cells. The presence of an enzyme exchanging potassium with sodium would be logical in the neurons, which ultimately have both to re-accumulate the potassium ions and to re-extrude the sodium ions which have been transported "down-hill" during stimulation. Also the $\text{Na}^+\text{-K}^+\text{-ATPase}$ has, however, a predominantly, though possibly not exclusively (Henn *et al.*, 1972) neuro-glial localization, and ouabain affects specifically glia cells.

In spite of the quantitative and ontogenetic differences between potassium effects on metabolism and on the $\text{Na}^+\text{-K}^+\text{-ATPase}$, stimulation of this enzyme

might be responsible for at least part of the potassium effects on ion and energy metabolism if potassium in addition to its effect on the ATPase exerted a regulatory action on other steps of the metabolic degradation which would then be rate-limiting for over-all metabolism during the activation of the enzyme. As a purely hypothetical possibility this might besides the ordinary reactions during glycolysis and the tricarboxylic acid cycle be the transport of metabolites, mainly glutamate from presynaptic mitochondria into the "non-axonal terminal" compartment suggested by Kerpel-Fronius & Hajos (1971) to be obligatory for complete oxidation in brain (VI A, 3b). A potassium effect at this step may be suggested by the potassium-induced release of glutamate (which may be most pronounced under anoxic conditions (V B, 1c) due to an efficient re-accumulation under oxygenated conditions), the absence of a potassium-induced release of glutamate neonatally (V B, 1c), the ability of low concentrations of lithium to inhibit both the potassium-induced release of glutamate (V B, 1c) and the stimulation of oxygen uptake (C. S. Kjeldsen & L. Hertz, unpublished experiments) and the ability of the same substrates to maintain a glutamate uptake and a potassium-induced respiratory stimulation. Such a mechanism would automatically transfer tricarboxylic acid cycle intermediates (and thus capacity for an increased metabolic degradation) from the axonal terminals to the structures involved in the termination of the stimulation by an active uptake of transmitters and potassium ions. The sodium requirement of the glutamate uptake might furthermore explain the gradual respiratory decline in sodium deficient media (II B, 1a) and in isolated glia cells (II B, 1b) and homogenates (II B, 1c) as due to a deficient supply of tricarboxylic acid cycle intermediates in the "non axonal terminal" compartment.

Conclusion

In the preceding chapters several indications, mutually supporting each other, were found that the potassium-induced stimulation of oxygen uptake (II), aerobic glycolysis (VI), and turnover of energy-rich phosphates (III) in the brain cortex is a metabolic manifestation of an increase in energy-requiring processes which probably includes transport of monovalent cations, especially potassium and chloride (IV), but possibly also transport or formation of certain amino acids (V), and is correlated with stimulation of potassium activated enzymes (VI). The synthesis of proteins and RNA may be slightly stimulated by a moderate increase in the potassium concentration, but the concentrations exerting pronounced effects on energy metabolism lead, in contrast, to an impaired synthesis of these macromolecules.

Almost general concordance is found that the metabolic response (II B, 2a; III B, 2a; IV B, 2b; VI B, 4c) and probably also a great fraction of the unstimulated energy metabolism (II B, 1b) occurs in the neuropil, whereas protein synthesis is mainly connected with nerve cell perikarya (V C). The close anatomical, ontogenetic (I B, 1 and 4) and possibly also metabolic correlation (VI C) between nerve cell processes and glia cell processes in the neuropil hampers, however, any unequivocal conclusion, whether primarily neurons or glia cells are involved. Both ontogenetic and histological evidence has been taken as support for the concept that glia cells, respectively neurons, possess the potassium stimulated enzyme(s) and react metabolically to deviations in the external potassium concentration. In the view of the present author, the bulk of evidence obtained from experiments in more highly developed species support the concept that it is the glia cells which mainly or exclusively are involved.

An uptake of potassium ions into glial cells would have considerable implications under both physiological (Trachtenberg & Pollen, 1970) and pathological

(e.g. Pollen & Trachtenberg, 1970) conditions, but the subsequent fate of the potassium ions and the mechanism of their obligatory re-accumulation into neurons (IV C) might have as great importance for brain function. In spite of a few observations (Orkand et al., 1966; Kuffler, 1967; Walker & Hild, 1969) or hypotheses (Hertz, 1965a, b) this remains a terra incognita, open for future research.

Summary

I. Aim of paper: Discussion of effects by deviations in the concentrations of monovalent cations on metabolism in the adult, mammalian brain with an attempt to correlate the effects exerted on energy-yielding and energy-requiring processes.

The histological complexity of the brain is emphasized with the resulting difficulties in experimental technique and interpretation of results.

II. The oxygen uptake by different preparations of brain tissue is compared. The concentrations of monovalent cations in the incubation medium affect respiration by cortical brain slices profoundly. A certain concentration of sodium is essential to maintain respiration and as a co-factor in the potassium-induced stimulation of oxygen uptake. Sodium may be replaced by choline in the former, and by lithium in the latter of these functions. High concentrations of potassium (or of certain other monovalent cations, including lithium), cause an increase in the rate of oxygen uptake above that observed during incubation in a "balanced" medium. The magnitude of the "stimulated" respiration is relatively close to the rate of oxygen uptake in vivo.

Only muscle resembles brain slices in showing an increased rate of oxygen uptake during incubation in potassium-rich media. So many differences are, on the other hand, found between the metabolic reactions by muscle and by brain to deviations in the ionic composition, that it seems unlikely that the same mechanisms should be involved. Within the nervous system the potassium-induced stimulation is confined mainly to grey matter from the rostral part. Histologically, its localization in glia cells is indicated by results obtained using different methods with different sources of error.

III. The turnover of acid-soluble phosphates (mainly ATP and creatine phosphate) is increased by excess potassium, and the concentration is decreased. This reduction is specific for glia cells.

Also acid-insoluble phosphorus fractions are affected by ionic deviations in the incubation medium.

IV. The exchange of potassium seems to be increased more than corresponding to the rise in diffusion when the concentration of potassium in the medium is increased. The raised efflux is compatible with the effect by a depolarization. Evidence is also found of a compensatory, probably active, uptake of potassium into glia cells together with chloride ions and water, but without any concomitant extrusion of sodium. Such an uptake of KCl and water might explain the potassium-induced increase in swelling, which shares several characteristics with the potassium-induced stimulation of oxygen uptake. It is accordingly suggested that an active uptake of KCl may be the energy-requiring process reflected by the potassium-induced increase in metabolic rate; such a mechanism might be of physiological importance in maintaining extracellular potassium homeostasis.

V. Transport and metabolism of amino acids are affected by deviations in the ionic composition of the medium. The synthesis of protein is mainly decreased by excess potassium and seems largely to occur in the neurons.

VI. Only certain substrates, i. e. glucose, pyruvate, lactate and oxaloacetate sustain a well maintained oxygen uptake which is increased by excess potassium. The other tricarboxylic acid intermediates do not give rise to a potassium-induced stimulation of oxygen uptake. Ketoglutarate and oxaloacetate lead to a better maintenance of the potassium concentration than of the sodium concentration in brain slices, supporting the concept of a dissociation between sodium and potassium transport.

The characteristics of the $\text{Na}^+ - \text{K}^+$ -ATPase and the pyruvate kinase are compared with those of the potassium-stimulated oxygen uptake. Many characteristics are common for the potassium effects on the ATPase and on oxygen consumption, but important differences seem also to exist.

VII. Conclusion: Evidence has been gathered that the potassium-induced stimulation of metabolism is a metabolic manifestation of an increase in energy-requiring processes which seem to include transport of KCl into glia cells.

Dansk resumé

I. Artiklens formål: Virkningerne af afvigelser i koncentrationerne af monovalente kationer på stofskiftet i den voksne pattedyrhjerne diskuteres, og det forsøges at korrelere de virkninger, der udøves på energigivende og energikrævende processer.

Der lægges vægt på hjernens histologisk komplicerede struktur og de heraf følgende vanskeligheder med hensyn til forsøgsteknik og fortolkning af resultaterne.

II. Iltoptagelsen i forskellige præparater af hjernevæv sammenlignes. Koncentrationerne af monovalente kationer i inkubationsmediet påvirker i høj grad respirationen i snit fra hjernens cortex. En vis koncentration af natrium kræves for at opretholde iltoptagelsen og som cofaktor under dennes stimulation med høje koncentrationer af kalium. Natrium kan erstattes af cholin ved vedligeholdelsen af respirationen og af lithium ved stimulationen af iltoptagelsen. Høje koncentrationer af kalium (og af visse andre monovalente kationer, inklusive lithium) forøger iltoptagelsen til et højere niveau end det, der iagttages under inkubation i et "balanceret" medium. Denne "stimulerede" respiration er af nogenlunde samme størrelse som iltoptagelsen in vivo.

Kun muskelvæv minder om hjernesnit ved at vise en forøget iltoptagelse under inkubation i kaliumrige medier. På den anden side findes så mange forskelle mellem virkningen af afvigelser i inkubationsmediets ionsammensætning på stofskiftet i muskel og hjerne, at det synes usandsynligt, at de samme reaktionsmekanismer skulle være involveret. Inden for nervesystemet er kaliumstimulationen stort set begrænset til grå substans fra dets rostrale del. Histologisk synes kaliumstimulationen at være lokaliseret til gliaceller, hvilket ses af resultater, der er opnået med forskellige metoder, behæftet med forskellige fejlkilder.

III. Omsætningen af syreopløseligt phosphat (hovedsageligt ATP og kreatinphosphat) er forøget i kaliumrige medier og koncentrationen nedsat. Denne nedsættelse er specifik for gliaceller.

Også syreuopløselige phosphatfraktioner påvirkes af afvigelser i inkubationsmediets ionsammensætning.

IV. Transporten af kalium ind og ud af hjernesnit forøges mere end svarende til den forventede stigning i diffusion, når mediets kaliumkoncentration forøges.

Den forøgede efflux er i overensstemmelse med, hvad man ville vente som resultat af en depolarisering. Som compensation herfor synes der at foregå en, sandsynligvis aktiv, akkumulation af kalium i gliaceller sammen med kloridioner og vand, men uden en tilsvarende ekstrusion af natrium. En sådan optagelse af KCl og vand ville kunne forklare den kalium-inducerede forøgelse af "swelling", som har mange karaktertræk fælles med den kalium-inducerede forøgelse af ilt-optagelsen. Det foreslås derfor, at en aktiv optagelse af KCl kunne være den energikrævende proces, som forårsager den kaliuminducerede forøgelse af stofskiftet. En sådan mekanisme ville være af fysiologisk betydning for opretholdelse af den ekstracellulære kalium homeostase.

V. Også aminosyrers transport og stofskifte påvirkes af afvigelser i mediets ionsammensætning. Protein syntesen viser hovedsagelig en hæmning, når kaliumkoncentrationen øges og foregår især i neuronerne.

VI. Kun visse substrater, d.v.s. glukose, pyruvat, laktat og oxaloacetat er i stand til at bevirke en vel vedligeholdt ilt-optagelse, som stimuleres af høje koncentrationer af kalium. Øvrige intermediære forbindelser fra trikarboxylsyre cyclus kan ikke bevirke en stimulering af ilt-optagelsen i medier med høje kaliumkoncentrationer. Ketoglutarat og oxaloacetat bevirker en bedre opretholdelse af kaliumkoncentrationen end af natriumkoncentrationen i hjernesnit, hvilket styrker opfattelsen af en dissociation mellem natrium og kalium transport.

$\text{Na}^+ - \text{K}^+$ -ATPasens og pyruvatkinasens egenskaber sammenlignes med den kaliumstimulerede ilt-optagelses. Mange træk er fælles ved kaliums effekt på ATPasen og på ilt-optagelsen, men der synes også at forekomme vigtige forskelle.

VII. Konklusion: Den kalium-inducerede stimulation af stofskiftet synes at være en metabolisk manifestation af en øgning i energikrævende processer, som sandsynligvis inkluderer transport af KCl ind i gliaceller.

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Index

(Numbers refer to present publication and letters to the previous studies listed in front of this publication.)

<u>Extracellular space:</u>	Extracellular space markers	I, 60, 73
	Potassium Homeostasis	C, 96
	Volume of extracellular space	14
<u>Glia cells:</u>	Electrical properties	68, 85
	Energy metabolism	D, F, 25, 32, 40, 48
	Ion metabolism	65, 81
	Methods for obtaining glia cells.....	D, L, 15
	Volume of glia cells	12
	Water metabolism ("swelling")	64, 76
<u>Nerve cells:</u>	Electrical properties	68, 85
	Energy metabolism	D, F, M, 25, 33, 48
	Ion metabolism	65
	Methods for obtaining nerve cells ...	D, L, 15
	Volume of nerve cells	12
<u>Slices:</u>	Amino acids	K, 102, 106
	Membrane potential	68, 85
	Oxygen consumption	A, B, N, 22, 34, 36
	Ion content	G, H, J, 64, 77
	Ion transport	E, 69, 89
	Spaces	I, 60, 73
	Swelling	G, 62, 74

